

More Interdisciplinary Theses

IN the past couple of years, academics in Britain have been urged by the Science Research Council to look at the style of postgraduate education provided by the Massachusetts Institute of Technology (MIT) and to think seriously about transplanting some of its best features to British universities. Particular attention has been drawn to the extensive course work done in the first year or two at the postgraduate level, the subsequent examination, success in which has been necessary before a thesis could be ventured into seriously, and the (by British standards) free-flowing interdisciplinary character of much of the work that a student would do. But how does MIT see its own intellectual future—is there to be more innovation in postgraduate education or is the present mixture regarded as about right?

MIT administrators see a variety of trends as both restricting and demanding future change. There is a general feeling that the shape of postgraduate education in terms of requirements is satisfactory and should not be tampered with. But the content is another matter; defence and space were the two big customers for MIT PhDs and for research projects at the institute in the 1960s, and this wave has now broken. Present day and near-future demands on science and engineering research are much more closely related to what one person described as 'ways of engaging reality', such as increasing industrial productivity, solving energy problems and looking at questions in biomedical engineering and in transportation. These are interdisciplinary problems *par excellence* and there seems general agreement that the interdisciplinary revolution at MIT has not yet run its course. There is a pretty clear lesson here for some of the narrow self-regarding departments in British universities.

New commitments to multidisciplinary research and education are bound, however, to come up against the severest of financial and administrative obstacles. A report last summer by an MIT committee charged with looking at the institute's research structure painted a generally gloomy picture of federal support levels which have stagnated for several years, and of the research environment, which many believed had declined in recent

years largely as a result of increasing pressure on faculty to raise research funds and a concomitant drop in flexibly-available resources. The committee endorsed the singular mix which MIT had evolved of departments, inter-departmental research programmes and specific laboratories and saw, in particular, research centres on campus as a way to proceed for the next decade; such concepts would be bound to influence the content of the education provided.

But if there are still new academic horizons, however shaky the funding prospects, there is one factor which is causing a lot of worry: changing levels of student enrolment in the next twenty years. Projections for the next ten years by the National Center for Education Statistics of the US Office of Education see a steady growth in bachelors degrees awarded in science and engineering up to 1985, with all the growth taken up in engineering, the life and the social sciences. But thereafter demographic trends take over. The number in the postgraduate age group (22–25) in 1995 will have dropped by 20% compared with 1985. This means not only that some inferior colleges will probably have insufficient students to justify their continuation, but also that even the big names in education will find themselves competing fiercely by 1990 to attract students to justify staffing levels.

And even before 1990 the PhD emerging from an American university is likely to find the world much changed from its present scene. Allan Cartter, in his book *PhDs and the Academic Labor Market*, projects US academic needs for junior faculty for the next twenty years. Whereas before 1970 academe could gobble up every PhD produced, by 1985 on the most optimistic projections (assuming faculty take early retirements and so on) there will only be an academic job for one PhD in every six produced in the United States. The moral is fairly obvious; discourage right now those who believe three or four years research is an admission ticket to a cosy faculty post somewhere; and go on broadening the scope of the PhD so that it becomes a very widely acceptable qualification. Hence the continuing talk of interdisciplinary postgraduate education. □



Assessing the Oklo phenomenon

Professor Alvin Weinberg, Director of Oak Ridge National Laboratory, Tennessee, comments on the lessons to be learned from the discovery of a natural reactor

THE Oklo River is a small stream that runs near the town of Mounana, Gabon; here there is a large, open-pit, uraninite mine which I visited a few months ago. Oklo has become famous ever since French scientists of the Commissariat à l'Énergie Atomique discovered in 1972 that the mine was the site of ancient spontaneous chain reactors some 1.8×10^9 years ago. At that time the uranium-235 concentration was about 3%, instead of its present 0.72%. This is the same concentration now used in the fuel of modern pressurised water reactors. Indeed, the reactors at Oklo operated for about 15,000 MW years—that is, the equivalent of 5 years of a modern 1,000 MW reactor—and reached a burn-up of about 25,000 MW days per ton of uranium, again about the same as is reached in a modern PWR.

The Oklo phenomenon is surely one of the most exciting scientific discoveries of the decade. I hope that it quickly becomes part of the common understanding not only of scientists, but also of laymen—just as *Pithecanthropus Erectus* or King Tutankhamen have become parts of the common, as well as the scientific, understanding. I am not unbiased in this desire. Despite all the fuss about nuclear energy, I am still an unabashed nuke. For me, the long-term alternatives to nuclear energy: solar energy (and its children—wind, waves, ocean thermal gradients, biomass), geothermal energy, and fusion are either too expensive (solar) or too small (geothermal) or too uncertain (fusion).

The path of conservation is all well and good for a developed country that has energy to conserve; it has little relevance for a country such as Gabon, or for that matter, most of the world that is bent on development. Nor can I take the talk about 'small is beautiful' or a 'fission-free bridge to a solar future' fully seriously. Has anyone demonstrated on a scale large enough to make the experiment convincing, that small is really beautiful rather than simply poor? Or can anyone speak so confidently about future heavy dependence on fossil fuel with the CO₂ in the atmosphere increasing at a rate of about 0.15% per year, probably because we are burning so much fossil fuel? No, it seems to me that nuclear energy will be necessary especially in the long run. The main job of the nuclear community at present

then is to make nuclear energy acceptable—to construct a nuclear future that adequately deals with the shortcomings of nuclear energy—some real, some only imagined.

We are all familiar with the issues by now: proliferation, reactor safety, terrorism and sabotage, diversion, waste disposal. Of these issues, there is little question that in the public's mind, and indeed, in the minds of most scientists outside the nuclear community, waste disposal is the most serious concern. As long ago as 1952, James Conant, the president of the American Chemical Society, asserted that the public would reject nuclear energy as not being worth the candle because the safe, permanent disposal of radioactive waste posed an insoluble problem. The recent Flowers report, on nuclear power and the environment, regards the problem of waste disposal as being in an unsatisfactory state. The new California law requires that a technology for permanent waste disposal must be demonstrated before reactors can be built there.

Until Oklo was discovered, permanent waste disposal in geologic formation seemed to be a problem that could not be solved in principle: since the wastes remain hazardous for thousands of years, one generation simply could not perform a definitive experiment. But Oklo has done much to change this. During the course of the chain reaction some five tons of fission

products and perhaps one-half this amount of plutonium and other transuranics were formed. Of the fission products, the gases, the alkalis and alkaline earths, and ruthenium disappeared. On the other hand, the rare earths and the plutonium, uranium, and presumably the other actinides, remained in place, within the limits of measurement (at least 80 per cent of the plutonium is accounted for). The strontium-90 probably decayed in place to zirconium-90; whatever leaching of alkaline earth fission products occurred took place over a time long compared to the 30-year half-life of the strontium-90.

Thus nature has performed the experiment, and the findings, on the whole, are reassuring. To be sure cesium-137, a soluble species, migrated from the site; on the other hand strontium-90 probably did not; and most important, the actinides, which pose the really long-term hazard (thousands rather than hundreds of years) remained in place.

Sceptics will still remain unconvinced; they will argue that Oklo represented a special geologic situation, that we cannot be absolutely sure—and that anyhow, there really is something new and spooky about this whole radiation thing. This is the essence of the matter. I shall never forget Enrico Fermi, during one of the monthly information meetings we held in 1943 at the wartime Metallurgical Laboratory, reminding us that the unique significance of the chain reaction lay in its bringing mankind in contact with unprecedented amounts of radioactivity. Never mind that radiation has become one of the best, if not the best, understood physical insults to the biosphere. It nevertheless represents something most people are unfamiliar with and are therefore afraid of.

Unless our society accepts the reality of radiation, and the proposition that radioactivity can be safely sequestered, nuclear energy will founder. And this is why it is so important that Oklo become a household word. Fermi was not the first to achieve the chain reaction, nor was he the first to put large, concentrated amounts of radioactivity on earth. Nature had achieved this 1.8×10^9 years earlier. Although the Oklo event affected life in the immediate vicinity, our planet survived and the biosphere evolved. Perhaps 100 years from now, when we have become fully accustomed to man-made chain reactors, we will look back and wonder why so much fuss was made about the problem of radioactive waste disposal which nature had already demonstrated to be tractable. □



The Oklo site

Grow your own grass

David Spurgeon examines a programme for improving agriculture in Antigua

A COMMON problem of Third World countries is an unfavourable balance of payments caused by a shortage of indigenous industry and a consequent reliance on imports from developed countries. This is particularly true of many of the small Caribbean islands, whose industries (when there are any) are often foreign-owned and more concerned with exports to an affluent overseas market than with self-sufficiency of domestic supplies.

The tiny, 108-square-mile island of Antigua is a case in point. Since a sugar cane company left the island some years ago, there has been little more than tourism and rum-making in the way of industry there—both the molasses for the rum and most of the beef for the tourists being imported. Where sugar cane once flourished, acacia bushes now grow like weeds. Yet much of this land, rocky and hilly though it often is, could be used for grazing cattle, and if only the grass and legumes were more abundant and of better nutritional quality, the island might become self-sufficient in beef and dairy production—and even export to other Leeward Islands.

This in fact is the aim of a research programme now being conducted in Antigua, Trinidad and Belize by the University of the West Indies (UWI) with the aid of a grant from Canada's International Development Research Centre. The programme began in 1972 and is now in its second phase. It has an Australian consultant, Dr Robert Burt of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), and the experiment is being watched with interest not only by the agricultural ministries of the three countries concerned but those of the neighbouring territories of Guyana, Barbados, Jamaica, Guadeloupe, Martinique and Cuba as well.

Two meetings have been held of representatives of agricultural ministries of the region—including representatives from other islands—to look at results where tests are being carried out with material from the project. The most recent was held in Antigua from 29 November–1 December. The Antigua component of the project is directed by a New Zealander, John Keoghan, who has taught at McGill's Macdonald College and done research at the University of Guelph. He is assisted by Belal Ahmed, a microbiologist from Bangladesh, employed by the department of soil science at the UWI in St Augustine, Trinidad, Clive Devers

from Guyana, a graduate assistant, and Perry Phillip, a technical assistant.

Interviewed in Antigua recently, Keoghan spoke of the implications of the project and the relevance of the Australian experience. All of the grasses growing in the Caribbean and thought of as 'native' to the area, actually come from Africa, he said, while the legumes come from the area itself. The soil in both regions of the world is heavy clay, and if work done in the Caribbean were applied to Africa it could revolutionise agriculture there.

"This is not far-fetched," he said. "This is what the Australians did. They went to South and Central America and found plants for Northern Australia, which is tropical. The legumes that dominate livestock agriculture in Northern Australia were originally growing as weeds in South and Central America."

There is a difference, of course. The Australians selected plants from acid soils for acid soil areas of their own. Keoghan and his colleagues collect plants from alkaline soils to match those of the dry areas where livestock graze both in the Caribbean and in Africa. So far, they have collected a total of about 1,000 grasses and legumes for planting in experimental plots, including 70 different varieties of grasses. They are carrying out a complete classification and description of the plants, and then are making an agronomic assessment under different growing conditions, for example with different types of soils, and in mixtures of grasses and legumes.

Two plots

Two experimental plots are being used, one of them near the old sugar mill, where the small amount of laboratory equipment required is also located, along with nursery facilities.

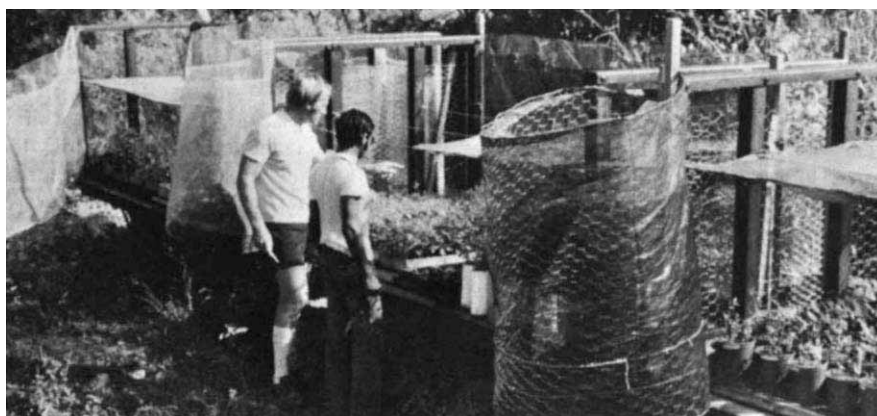
As basic as all this might seem to be, it has never been done before, according to Keoghan. Although there were earlier sporadic attempts at collection, classification and description, adequate records were not kept of them and plant collections disappeared.

Pasture legumes are important not only for their high protein content, but for their ability to fix nitrogen and thus act as a fertiliser for the grasses. This latter capability is all the more important to developing countries at a time of high fertiliser prices. Samples of legumes have been collected from all over the island as well as from other countries in the region.

Samples are selected from a six-square-metre area of each plot, the leaves are clipped and the yield is bagged, dried in ovens at the sugar factory and the weight measured. Nitrogen and protein content are determined, and records are kept of these measurements. Seeds are collected and examined, and then sent to CSIRO in Australia, which is the main centre of tropical legume research.

After two years of trials in these plots, the research group in Antigua is now beginning to mix the best grasses and legumes in field trials on heavy soils, and then will test them under actual grazing conditions. The types of soil used for such trials is representative of those found in much of the grazing area in Antigua. This is being done with 15 different grasses and 12 legumes, both singly and in mixed stands to determine the effects of the legumes and grasses on each other. The animal grazing tests will show whether, for example, the animals actually will eat the plants, and how well the plants grow again after grazing.

Basically, the idea is to narrow down the choice of grasses and legumes to determine the best varieties available for local conditions, and the most productive combinations when grown together, and to work out the most suitable management practices. It is the first time such a project has been undertaken in the Caribbean. □



Pasture legumes in moveable nursery made from railway cars

Scottish science and devolution

Martin Goldman looks at possible effects of devolution on science in Scotland

FACED with the present media overkill of the devolution debate, more and more Scots are displaying a traditionally 'British' response to the issue—apathy. At the risk of increasing the tendency, Scottish science deserves special attention, for the problem of reconciling the international horizons of science with the nationalist viewpoint is of wider relevance, even though it is still largely theoretical. The Scottish National Party, the spearhead of the independence movement, has not yet needed to produce a concrete science policy. They have not decided whether nuclear power is an invention 'o'er the de'il', much less what role it would play in an overall energy strategy, and have not even considered whether or how to continue as members of CERN.

More positive has been the concern of academics. A strong feeling against the devolution of Scottish universities was displayed in response to a questionnaire sent out by the Scottish Association of University Teachers. About 60% of the replies disapproved of devolution: 20% approved, 20% were neutral.

A paper on the whole subject presented to the government by Professor John Gunn, a theoretical physicist, and Andrew Thomson, an economist, gives the consensus view. They feel that universities ought to be international in outlook, and therefore anything hindering communications between British universities, especially the easy exchange of staff and students, is a bad thing. The decline of the international reputation of Trinity College, Dublin, since Irish independence is an example.

Their other major argument is that there exist economies of scale. Each university department has particular areas where it focuses its attention; Scottish universities do not cover a complete academic spectrum and are overproductive in certain areas (for example, Scotland, with 10% of the UK population, produces 150 vets a year out of a UK total of 350). Because of the expense of equipment, particularly in the sciences, and the existence of critical thresholds on the size of department, below which useful research is not done, such specialisation makes good sense.

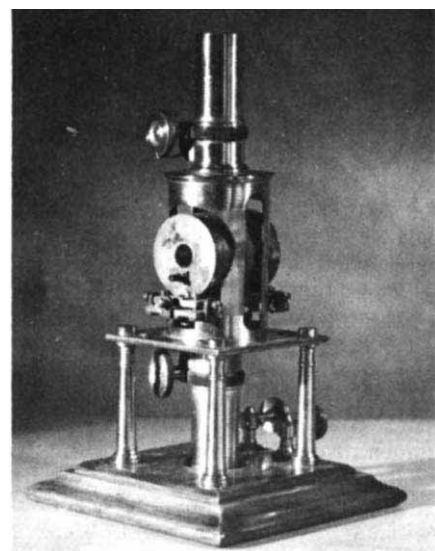
In addition to these general arguments, they add that the present funding system, through the Universities Grants Committee (UGC) and various research councils, works well in that it

allows a large degree of autonomy to the universities. If a Scottish UGC were created, it would be a cosier affair than the British one, and 'inevitable incestuous relationships' would ensue. Equally dreaded would be the alternative of integration with the Scottish Educational Department (SED) responsible for the present Scottish educational system. Universities are, of necessity, elitist organisations and are afraid they would be swamped if included with other forms of education. In addition the track record of the SED is appalling. In spite of (or because of?) a new newmaths syllabus, there has been a catastrophic disintegration of mathematical education in Scotland. All Scottish university science departments complain bitterly of the mathematical illiteracy of incoming students—the educational elite, no less—who cannot cross-multiply fractions, know no trigonometry, and require remedial courses to teach them basic skills.

Dissenting view

These views contrast strongly with those of both the SNP and the Scottish Union of Students. At the same time as Gunn and Thomson argued their case, Professor R. S. Silver, an internationally renowned expert on desalination and a life-long nationalist, presented a dissenting view. On the 'internationalist' argument, he contends that other small countries such as Holland and Israel have solved the problem. An independent Scotland would be forced to look out more to the world. He repudiates the inevitability of incestuous relationships, arguing that if this were so, then conversely Russia and China would have the fairest support system in the world. Professor Silver also points out that estrangement will inevitably arise under the present proposals between the institutes of technology (controlled from Edinburgh) and the universities (controlled from London). As for the economy of scale argument, he thinks that 'big science' has already grown too big. It leads to massive engineering projects that are socially destructive and tie together the world's economies in an unstable manner, like a house of cards.

Since then the debate has descended to the letter columns of *The Scotsman* and the *THES*. John Hulbert, prospective SNP MP and former lecturer in bacteriology at the University of Dundee, has waded in with his analysis.



*Produce of Scotland:
Kelvin's mirror galvanometer*

He finds it 'sinister' that since 1939 the proportion of professors in Scotland with a Scottish education has dropped from 73% to 48%. Remembering that Scotland has only 10% of the UK population, most would find this evidence of continued parochialism, and his views an unhappy auguration of future SNP thinking on the 'internationalism' of Scottish universities.

To Professor Malcolm Slesser of the energy studies department of Strathclyde University, who is also a nationalist, this idea of 'technological ecology' is also central. Engineering should be on an appropriate, human scale, and vast expensive projects should be eschewed. He, for one, would not complain if an independent Scotland could not build the Concorde. Silver and Slesser's argument is weakened by the fact that whatever the final scale of a project, the cost of developing even appropriate technologies is now vast, as exemplified by the programmes afoot in the USA, France and Japan to utilise solar power. Equally, almost no science is conceivable nowadays without a big computer. How big a computer could Scotland afford?

Professor Slesser thinks the real problem of Scotland stems from a lack of financial support for the development of appropriate new technology. He would like to see a revival of the technological entrepreneurship that characterised nineteenth century Scottish science, when a scientist such as Lord Kelvin could make a fortune (and get knighted) from his inventions. An investigation of government spending supports his view. Scotland does get a fair share of the UGC's and the research councils' university spending. The research councils also support special units: the MRC supports many all over the UK, and Scotland does

rather well, with 11 of these. The MRC works on the human scale, however; the SRC, which is responsible for 'big science', believes it has to have its expensive equipment housed in central facilities, shared by the country as a whole. This argument is rather damaged by the fact that its major 'centre', consisting of the Rutherford Laboratory (particle physics), the Culham Laboratory (plasma physics), the National Computer Laboratory, the High Power Laser Facility and the Appleton Laboratory (space and atmospheric research), is very conveniently situated for London, but is well south of the academic centre of the UK, which is strongly weighted by the Northern and Scottish universities.

This is a mere peccadillo compared to the policy of the biggest research spender of the country, the Ministry of Defence. Of the 45 major research establishments listed by the MOD, apart from the lonely Naval Construction Research Establishment in Dunfermline, none are farther north than Baldock, 35 miles from London. As part of the devolution package the government has promised to move the MOD to Glasgow, which has a chronic unemployment problem. Every few weeks Glaswegians read in their newspapers, with a mixture of anger and amusement, the ideas of civil servants on the putative great trek North. The most common view seems to be that civilisation stops at Chingford and the Arctic Circle begins at Watford Gap.

Hopefully, if the MOD is ever moved it will learn the error of its views and devolve some of its research establishments also.

Eternal drawback

What, then, about industrial research? While the government tries to encourage industry to the North with various short term benefits, there is an eternal drawback in that Scotland is at the periphery of Europe, and transport costs must always be high. This adds about £50 to the cost of a car made in Scotland rather than Birmingham. The more valuable the basic product, however, the less significant are these transport costs, and thus factories producing high technology items are least affected. This has been recognised by the American computer giants, and Honeywell, IBM, NCR, and Hewlett Packard all have factories in Scotland. They are just factories, however, and the best Scottish managerial and research personnel are siphoned off to England or abroad. Could the process be reversed by independence? This seems doubtful, in that a small Scotland is unlikely to be more successful at dealing with a high-technology multinational than the UK as a whole.

The lack of investment in new technology is also borne out by the experiences of the National Engineering Laboratory (NEL) at East Kilbride. Seven years ago the Rothschild committee recommended that the NEL should become less academic and liaise

more with industry—should become, in effect, East Kilbride's answer to MIT. To a certain extent this has worked: of an annual budget of £10,000,000, industry now contributes £1,250,000, and of NEL's marketable ideas about half are taken up by local Scottish industry—eventually. John McAllen of NEL's industrial marketing unit is, however, depressed at the lack of interest that most of industry displays in trying to identify areas where new technology could be developed. Especially depressing is the tale of NEL's hydraulically driven dumper truck. In spite of a total lack of industrial interest NEL were convinced that this idea had promise, and developed it on their own. Their perseverance paid off, and eventually Carron Hydraulics of Falkirk took out a franchise on the invention. When the potential of the idea was proved, however, Carron Hydraulics was promptly taken over by the German Rexroth group.

The story of other countries reaping the rewards from British inventions is now only too familiar. The worrying aspect of this case is that the invention was developed with government money specifically to benefit British industry; safeguards against subsequent foreign takeover should have been written in. The NEL is, nevertheless, of great importance to Scotland, both as an employer of scientific personnel and as a stimulus to industry. Its status in an independent Scotland would be very doubtful. □

USA

Maintaining momentum

Colin Norman reports from Washington on latest developments concerning recombinant DNA research

THE debate over the risks and benefits associated with recombinant DNA research, which has been raging in college campuses and city halls around the United States, suddenly picked up momentum in Washington last week. At one end of town, a public meeting sponsored by the National Academy of Sciences provided a forum for a clash of views on whether, and under what circumstances, such research should be permitted. At the other end of town, Representative Paul Rogers, chairman of the House health subcommittee, proposed legislation to regulate all recombinant DNA research in the United States. And in suburban Bethesda, a committee of officials from several government agencies met to try to draft a bill to be sponsored by the Carter Administration.

About the only clear message to emerge from this flurry of activity is that legislation to control recombinant DNA experiments throughout the United States is now inevitable, and that it would be welcomed even by many of the scientists who have long shivered at the thought of having an agency of the federal government regulate their research. But there was the glimmering of another message, at least in the Academy's public meeting. There emerged at the meeting vocal opposition to recombinant DNA research from non-scientists concerned about its potential long-term implications for human genetic engineering. Most of the debate so far has swirled around immediate health hazards, but it may slowly be shifted toward a discussion of the potential uses to which the research may ultimately be put.

First, the legislative developments. The National Institutes of Health (NIH) guidelines published last June

formally apply only to recombinant DNA research supported by the federal government, and are not backed by any federal monitoring or enforcement mechanism. Industrial research is not formally regulated at present. Because of these apparent weaknesses some state and local governments are considering adopting their own enforceable regulations. Faced with that prospect, many scientists who once resisted federal legislation would now welcome it.

Whether scientists want it or not, however, legislation is definitely coming. For the past four months a task force of some 25 officials from federal agencies has been seeking ways to extend the NIH guidelines to cover all recombinant DNA research in the United States and to monitor compliance with the guidelines. The task force has recently concluded that no existing federal agency has the authority to carry out those roles, and that new legislation is therefore needed.

The task force met last week to try to draft a bill for approval by the Secretary of Health, Education and

Welfare (HEW), who would then submit it to Congress. The task force didn't complete its work, however, and it will meet again this week. Its draft legislation is expected to recommend the establishment of a licensing scheme, administered by an agency of HEW (possibly the Center for Disease Control), for research involving recombinant DNA. In any case, the controls to be enforced on the research will be the NIH guidelines.

Meanwhile, Representative Paul Rogers has already got off the mark with a bill of his own. On 8 March, he made a speech in the House outlining

some of the risks and benefits potentially arising from recombinant DNA research, and announced that he would introduce legislation to control the research and that his health subcommittee would hold public hearings on the measure on 15, 16 and 17 March. The following day, he introduced a bill co-sponsored by nine other members of his subcommittee.

The timing of the move was based on political considerations. The House Committee on Science, Research and Technology announced last week that it would hold a series of public hearings on recombinant DNA research during

the last week of March, and Senator Kennedy's Senate health subcommittee is planning to hold hearings early in April, after the Administration's bill is introduced into Congress. Rogers didn't want to be in the position of seeming to react to events, so he had his staff draft a bill last week in record time.

When he introduced the bill, Rogers noted that "this proposal is intended to be a vehicle for discussion . . . and does not necessarily represent the views of its co-sponsors." Its chief provisions are as follows:

- It would require the Secretary of HEW to issue regulations governing recombinant DNA research which would be "no less stringent than the physical and biological containment requirements prescribed by the (NIH guidelines)". The regulations would also specify a role for institutional review committees.

- Any researcher wishing to conduct recombinant DNA experiments would have to obtain a licence from HEW, the application for which would describe the experiment and the conditions under which it would be performed. Licences would be valid for a maximum of two years.

- The bill would limit the number of high-containment (P4) facilities in the United States to ten centres.

- It would require the Secretary of HEW to publish in the *Federal Register* a description of projects for which licences have been granted, though trade secrets would be exempted from public disclosure.

- The Secretary would be empowered to inspect facilities in which recombinant DNA research is conducted, to ensure compliance with the regulations.

- Violations of the regulations would result in civil fines of \$1,000 per day.

- The bill specifies that federal regulations would pre-empt local controls on recombinant DNA research, except that the Secretary could exempt local controls which are at least as strict as the federal regulations.

- Finally, it would establish an advisory committee to assist the Secretary in the granting of licences for recombinant DNA research.

Last month a bill establishing a licensing scheme and providing harsh penalties for violations of the regulations was introduced by Senator Dale Bumpers and Representative Richard Ottinger. Senator Kennedy may also draft a bill of his own. The final version to emerge will be a compromise between many different proposals, though particular attention should be paid to the Rogers bill if only because he is in a key position to steer his proposals through the House. A recent notable addition to Rogers' subcommittee staff is Burke Zimmer-

In an action which has so far attracted little public attention, a biologist at the University of California has been forced to destroy a large and painstakingly constructed clone bank because the clones were produced under conditions disallowed by the NIH guidelines governing recombinant DNA experiments. The incident has been described by some scientists as a good example of the way that the voluntary NIH guidelines can work to protect public health, but others have suggested that it underlines the need for more careful scrutiny of recombinant DNA research proposals before experiments are begun.

The research, carried out by John Carbon of the University of California at Santa Barbara, was performed between mid-1974 and mid-1976, a time when the NIH guidelines were under development. During most of that period, recombinant DNA research in the United States was governed by a loose set of safety guidelines drafted by a group of scientists which met early in 1975 at Asilomar, California. Those Asilomar guidelines did not rule out Carbon's experiments and even the final NIH guidelines, published on 23 June 1976, are a little ambiguous on the matter. Carbon thus did not infringe guidelines in force while he was conducting the experiments and he says he was unaware that his work lay outside the final NIH guidelines.

The work consisted essentially of constructing a large bank of clones of *E. coli* containing transplanted yeast genes and fruit fly genes. Taking the clone bank of yeast genes as an example, this, in short, is what Carbon did:—

He extracted the entire nuclear DNA from yeast cells and chopped it up into small fragments by mechanical shearing. He then spliced the yeast DNA fragments into bacterial plasmids and inserted the hybrid plasmids into *E. coli*. The next step was to culture the bacteria, growing several thousand distinct colonies, and each colony was then harvested separately. The collection of colonies constitutes a clone bank containing copies of DNA fragments, representing the entire yeast genome, spliced into plasmids inside living bacteria.

None of that would ordinarily be considered hazardous; indeed, the NIH guidelines specify that such manipulations with yeast genes require only low (P2) levels of physical containment. The problem, however, was that the *E. coli* which Carbon used to clone the hybrid plasmids also contained so-called fertility, or F, plasmids which promote the transfer of genetic information from one bacterial strain to another. This means

that the hybrid plasmids contained in the *E. coli* might easily be transferred into other *E. coli* strains capable of surviving in the natural environment. In other words, use of bacteria containing F plasmids greatly lowers the barriers preventing escape of recombinant DNA molecules from the laboratory.

The scientific advantage of the system, however, is that it can be used for rapid identification of the transplanted genes, and it can also be used simply to test whether they are capable of being expressed in their new host. Essentially, Carbon transferred the plasmids from the *E. coli* bank into other strains and tested to see whether the properties of the new host were altered. In a paper published in the February issue of the *Proceedings of the National Academy of Sciences*, for example, Carbon and his co-workers report evidence that yeast genes are being expressed in their new hosts.

Last June, a couple of weeks before the NIH guidelines were published, Carbon presented the results of his work at a symposium on recombinant DNA held at Massachusetts Institutes of Technology, and his use of a system containing F plasmids was challenged on grounds of safety. Then, shortly after the NIH guidelines were issued, Carbon wrote to the NIH Recombinant DNA Advisory Committee for a ruling on whether or not his system is proscribed by the guidelines. The committee informed him last October that the guidelines should be interpreted as ruling out the use of *E. coli* containing F plasmids, and it instructed him to destroy his clone banks. Carbon was, however, given time to extract those plasmids which he wished to keep and transfer them to safer strains. He complied with the ruling last November.

Asked last week when he first had doubts about whether his experiments infringed the guidelines, Carbon said that "the realisation dawned on us slowly over the course of several weeks last spring". He said, however, that the guidelines do not specifically rule out use of his system, and he was therefore still uncertain about the propriety of his experiments even when the guidelines were issued. Other scientists queried last week said that although the guidelines are a little fuzzy on the matter, they thought that it was generally assumed that *E. coli* with F plasmids shouldn't be used. In any case, Carbon said that he is "willing to go along with the guidelines" and he destroyed more than 50,000 clones containing yeast genes and some 10,000 clones containing *Drosophila* genes.

man, a former representative of the Environmental Defense Fund who has long been active in seeking more public debate and stricter controls on recombinant DNA research.

While those legislative developments were taking place last week, the Academy's public meeting was in full, and sometimes noisy, swing at the other end of the Mall. Though the meeting essentially covered ground which has already been well trodden in college campuses and city halls around the country, it nevertheless generated considerable national attention and not a little friction.

Most of the high-temperature exchanges took place at the opening session, when a group called the Peoples' Business Commission (a reincarnation of the Peoples' Bicentennial Commission) staged a series of disruptions. Jeremy Rifkin, a spokesman for the group, was invited to address the meeting to air his grievances, and as he did so several of his supporters unfurled banners proclaiming their opposition to the research. Rifkin harangued the organisers of the meeting, claiming that the fact that it was supported financially by several drug companies rendered it suspect. In particular, Rifkin claimed that because the agenda was dominated by sessions concerned with potential health hazards from recombinant DNA research, it missed the central question of how the long-term implications of the research should be dealt with. Genetic engineering, he opined, is "the most important issue that society has to grapple with", and he told the researchers present that "you can't hide from the facts any more than the physicists who split the atom".

Although the interruptions and objections formed just a part of the meeting, Rifkin's objections did surface later in rather more subtle form, from speakers such as Jon Beckwith of Harvard, who noted that though there are still some barriers left to introducing genes into human cells, "these goals are not at all inconceivable and they may be achieved very rapidly". Beckwith announced that he has renounced use of recombinant DNA techniques, because "I do not wish to contribute to the development of a technology which I believe will have profound and harmful effects on this society".

Other speakers noted that one of the potential benefits often claimed for recombinant DNA is to help understand the causes of cancer. Such observations put the debate onto a different level from discussion of potential health hazards, and they could be much more difficult for scientists to grapple with. □

Sweet sorrow

AFTER more than six years of uncertainty, the Food and Drug Administration (FDA) last week decided to ban the artificial sweetener saccharin from general use in the United States. The action, which was prompted by clear evidence that the sweetener can cause bladder cancer in rats when fed to them in large quantities, immediately prompted shrill protests from the food industry, and it is sure to touch off a new round of attacks on the so-called Delaney Amendment which bans use of any food additive found to cause cancer in animals.

The evidence which finally sounded the death knell for saccharin came from a long-term feeding study sponsored by the Canadian government, preliminary results of which were made available to FDA officials on 7 March. Two days later, FDA and the Canadian Department of Health and Welfare announced that the sweetener would be banned both in Canada and the United States.

The full results of the study have not been published, but according to a summary made available by FDA, it turned up the following results. Out of 50 male and 50 female rats fed a diet containing 5% saccharin throughout their lives, three males and no females developed malignant bladder tumours. In addition, 50 male and 50 female offspring of those rats were also fed diets loaded with saccharin. The cancer incidence among them was more pronounced: 12 male and 2 female animals developed bladder tumours. Thus, 17 out of 200 test rats developed cancer compared with 2 out of 100 in a control group.

Until the full results are published, it is difficult to tell how good the study was. It has, however, long been regarded as the best test of saccharin yet undertaken, and FDA officials have been eagerly awaiting the results in the expectation that the study would finally settle the question of whether saccharin is or is not a carcinogen. They were impressed. The tests, according to Sherwin Gardner, the Acting FDA Commissioner, "show unequivocally that this substance can produce malignant bladder tumours in rats".

It has sometimes been suggested that the carcinogenic potential of saccharin may be caused not by the sweetener but by an impurity, ortho-toluenesulphonamide (OTS). The Canadian tests, however, included a separate study of OTS and no carcinogenic effects were discovered.

FDA was therefore left with no choice but to ban saccharin from the market, an action which Gardner suggested was "based on science and on the requirements of Federal law". FDA hopes to publish details of the ban within a month, but it will allow those products already manufactured to be sold.

The ban could have considerable economic impact since saccharin is now the only artificial sweetener on the market in the United States. Cyclamates were banned in 1969, following evidence that they also cause bladder cancer in rats. And another sweetener, aspartame, was due to be approved by FDA a couple of years ago but approval was withdrawn following a discovery that the manufacturer of the substance submitted misleading test data. Once saccharin is removed from the market, there will be no non-nutritive sweetener for use by the burgeoning diet food industry. Some 5 million pounds of saccharin is now used in the United States, about three quarters of which goes into soda pop.

FDA's regulation of saccharin came under blistering attack last year in a study published by the General Accounting Office (GAO), an investigatory agency of the Congress. Noting that doubts about the safety of saccharin were raised in the early 1970s, particularly after a study published in 1971 indicated that the sweetener may be a potential carcinogen, GAO suggested that "extended use of a food additive, such as saccharin, whose safety has not been established and for which a carcinogenic potential has been raised, could expose the public to unnecessary risk".

FDA did, however, take some action against saccharin in 1972. It limited the amount of the sweetener which could be added to food and drink, and ordered that the substance should be used only in special dietary products. The latter requirement hasn't been rigorously enforced, however, for use of the sweetener has mushroomed, diet sodas are heavily promoted for general use, and saccharin is now found in products like toothpaste and pancake syrup.

With such a large volume of sales to protect, the food industry is expected to appeal the ban, through the courts if necessary. Some nutritionists are warning of the health consequences of an increased consumption of sugar.

Colin Norman

USSR

● The Soviet media have recently been devoting considerable attention to peaceful nuclear explosions (PNE). Although the Soviet PNE programme dates back to 1965, so far explosions have been carried out without publicity. Recently, however, a number of Soviet press and radio reports have extolled the use of PNE in digging the Pechora-Volga canal, and the construction of water and underground gas storage reservoirs. An interesting feature of this campaign is that all three major broadcasts on PNE achievements were in English (one being in the African service), and therefore aimed at the outside world; in one case, the contents of the broadcast was then issued in a *Novosti* press release.

Soviet experts do not share American doubts on the commercial viability of PNE, and are eager for the ratification of the PNE agreement, already initialled in Moscow and awaiting ratification by Washington, which would ensure mutual on-site inspection and instrumentation rights. Although the Soviet delegate to the Geneva disarmament talks, Mr V. A. Likhachev, recently called for a total cessation of all nuclear weapon tests, there are clear indications that the Soviet Union would not be willing to accept a ban or a moratorium on PNE. The recent burst of publicity may well be intended to make the Soviet attitude clear to those governments which would favour a total ban on all nuclear explosions.

● Conservation of the country's resources, including energy, is an important feature of the present Five-Year Plan. Targets include savings of 14-16% ferrous metal in machine construction, 5-7% metal, 5-6% cement and 12-14% timber in building, and 5% electrical and thermal energy and 8% petrol and diesel fuel.

This winter, inspections and surveys have been made in many parts of the Soviet Union to monitor fuel consumption. A supporting campaign in the media, commending the economical consumers and rebuking the wasteful, is aimed at all levels of the community—from the electrical generating industry, which last December cut its specific consumption by 2 g fuel per kW-hour (saving some 1.5 million tonnes of coal), to the workers of Vladivostok, who by their "thousands of valuable suggestions" have allowed some 13 million kW of electricity and 49,000 tonnes of rated fuel to be saved.

Particular criticism has been directed at the Ministry of Fuel and

Power (for slowness in converting to more efficient boilers), at the metallurgical industry (for poor use of recycled resources), and the Ministry of Construction of the Oil and Fuel Industries (for delays in constructing gas refineries, so that "thousands of millions of cubic metres of gas" have had to be burned off into the atmosphere) and various local authorities (for lighting and heating empty build-



ings, using expensive fuel from elsewhere rather than cheap local coal, peat or wood).

● Three Moscow refusniks, the cyberneticist Aleksandr Lerner and Vladimir Slepak and Anatolii Shcharanskii, both engineers, are attempting to bring a libel suit against Dr Senya Lipavskii and the editor of the government newspaper *Izvestiya*. Dr Lipavskii, a former associate of the refusniks, is the author of an 'open letter' published in *Izvestiya* alleging that a number of the prominent Jewish activists, including the three men, are CIA agents.

Such denunciations have in the past always been followed by criminal proceedings, and it would be unprecedented if no action were to be taken in this case. Shcharanskii, who often acts as unofficial spokesman for the group, has already had all his documents except his internal passport confiscated. To try to refute the expected charges, Lerner, Slepak, and Shcharanskii have attempted to initiate proceedings for libel, although various bureaucratic barriers are being placed in their way.

Together with the economist Ida Nudel and Dina Beilina, an engineer, they have also written a letter to President Carter, asking him to do something "constructive" to ease the situation. Although the text of the letter has not been released by the

signatories, it seems that they would urge a firm but 'soft' response. The letter of Academician Sakharov to President Carter last January, which called on the President to "raise your voice" against human rights violations in the Soviet Union, was interpreted by the Soviet authorities as a provocation to cold war, as was the recent meeting of the President with Vladimir Bukovskii. Lerner and his friends, it would seem, are unwilling that their case should be used to undermine the very real benefits of détente.

● The new Soviet 200-mile fishing zone, which came into force on 1 March, has inaugurated a new era in Japanese-Soviet fishing relations. So said the Japanese Minister of Agriculture and Fisheries, Zenko Suzuki, during four days of stiff negotiation in Moscow at the turn of the month. By the end of the talks it was clear that the new era was not to be one of immediate agreement: indeed, Mr Suzuki and the Soviet Minister of Fisheries, Aleksandr Ishkov, could not even agree on a joint communiqué, and the talks ended in a formal exchange of notes.

During the talks Mr Suzuki stated that for constitutional reasons Japan could not accept certain provisions of the decree establishing the Soviet zone. His concluding note to Ishkov stated that Japan would also be establishing a 200-mile zone in the near future. This will encroach upon the new Soviet zone on the west coast of Japan and in the region of the La Pérouse and Kunasharskii straits. In spite of these diplomatic difficulties, however, certain practical conclusions were reached, pending the interim pact (expected by 31 March). Japanese fishing vessels will be permitted to operate within the new Soviet zone, but may not engage in herring or salmon fishing. Negotiations are to be continued towards a long term agreement.

A similar position, but with the Soviet Union in the role of the applicant seeking to enter an exclusive zone, has arisen in the fisheries talks between the Soviet Union and the EEC. The first round of talks, in February, were hailed as a diplomatic breakthrough; the second round failed this month to produce the "mutually acceptable results" which Mr Ishkov had hoped for. Since both sides realise that they are at present too far apart to reach an early agreement, the talks have been postponed until 19 April.

Vera Rich

BRITAIN

● Figures on graduate employment from three of Britain's top universities don't conform with everyone's predilections when it comes to science graduates.

The view heard these days is that graduates, especially from Oxford, Cambridge and London, are likely to go into the types of employment which served Empire well but which are of less obvious use in the modern industrial era. The suggestion is that they are most likely to go into central and local government or teaching when they aren't busy becoming perpetual students by doing post-graduate study. Otherwise they are likely to be joining the professions—becoming accountants or solicitors, moving into banking or insurance—or joining the media by going into journalism, broadcasting or publishing. What they aren't doing, the argument goes, is helping to boost Britain's long term economic potential by going into directly productive employment in industry.

Figures giving details of employment of 1976 science graduates from Oxford, Cambridge and London do not appear to bear these assertions out. Even after making allowances for difficulties in interpreting the statistics in precisely comparable terms, the indications are that Britain's graduates in the pure sciences, if they are not going into research, are most likely to go into industry.

That they are likely to go on to further study is strongly indicated by the figures for both Oxford and Cambridge, where a high proportion, around one-third, of the men science graduates from both places opted for this course. But of the 390 Oxford men science graduates who worked in other fields in Britain after graduating, 155 went into industry; and of the 193 Cambridge science graduates, 88 did so. For London, the figure was 209 out of 497.

For the public service and education, moreover, the figures are not high. Just 20 out of 193 from Cambridge went into the public service, and 23 directly or indirectly into teaching. The comparable figures from Oxford are 65 and 44 (out of 390) and from London 75 and 68 (out of 497). Similar tendencies are apparent in figures for accountancy (32, 58, 47) and for banking and insurance (7, not available, and 34). And from all three universities together a total of only 21 men went into the media or elected to become solicitors.

What makes the figures even more interesting is that similar trends, though less marked, can be identified

for arts and social studies graduates. At the other end of the university machine, meanwhile, the number of entrants into tertiary education who go into science and engineering remains a prominent topic, not least, it seems, because the facts are disputed. At the beginning of the month the Education and Science Secretary, Mrs Shirley Williams, described the need to attract students into science



and engineering courses as "urgent". Her department is at the moment investigating whether to introduce a form of scholarship to encourage sixth-formers to specialise in science and engineering. But the Vice-Chancellor of Durham has warned of a glut of engineers, and the London University Institute of Education is saying the shortage of engineers and scientists is a myth.

● The state of Britain's manufacturing industry is an issue unlikely to be superseded in importance by questions about her agriculture, itself an industry. At the end of last month, though, it was announced that the country's standing Royal Commission on Environmental Pollution, now under the chairmanship of Professor Hans Kornberg, was to examine how the trend towards large livestock units and the greater use of fertilisers and pesticides posed an increased risk of pollution. The Commission also wants to examine the vulnerability of agriculture to industrial pollution.

Among the bodies which might show an interest is the Nature Conservancy Council (NCC). A report it published last week suggests that financial assistance and advice on conservation should be given to farmers if the problems their farming practices create for wildlife are to be alleviated. The report estimates that if all farms were to be totally modern-

ised, about 80% of the bird and 95% of the butterfly species would be lost from the farmed landscape. The NCC wants a policy of wise land use which provides for the conservation of wildlife as well as increased food production.

Increased food production was just one of the points broached last week by Professor John Bowman, the Director of the Centre for Agricultural Strategy at the University of Reading, when he spoke about an agriculture strategy for Britain. He talked of food consumption ("comfortably in excess of or equivalent to recommended minimum levels for normal life"), of changing eating habits to help feed others less fortunate (that had "little to commend it"), curtailing animal production, because of worries about its cruelty or its energy inefficiency ("it is more justifiable to argue for the modification of production systems so they are based more on feed inputs of no direct use to man"), and justifying protectionist policies in terms of security (there are sound arguments for achieving security "through an increase in the world trade of all commodities, not least of which are food commodities").

He also presented the preliminary findings from the Centre's own model of the UK agricultural economy. Its projections for a future with a higher degree of self-sufficiency and lower import costs indicated broadly that, even allowing for modifications in diet, meat production was expected to contract, the dairy sector, sugar beet and oilseed rape were expected to expand, and the cereal acreage would remain largely unchanged. But technical and economic efficiency were unlikely to be the only objectives of public policy; justice, equity and security could take precedence.

● The University of Bradford's Disaster Research Unit, which was established in December 1973 and has just three members, is now fighting for its survival. When the initial period of three years for which the unit was financed came to an end late last year, the university (which provided the funds along with the Leverhulme Trust and the Ministry of Overseas Development) agreed to finance a further year pending the outcome of a review. This showed that with the cuts it faced the university could not support the unit. Now the unit has stopped work while its members ask the government, international agencies and trusts for more funds.

Chris Sherwell

BRITAIN

Potential energy

Developments on Britain's energy front are continuing apace. Chris Sherwell reports

GAS, coal, oil and nuclear power have all featured in the past week's energy developments. At the risk of involving the Cabinet and angering the unions, the Energy Secretary, Mr Anthony Wedgwood Benn, is proposing to overrule the Price Commission's rejection of the British Gas Corporation's (BGC) application for a price rise. The rise, effective next month, will boost BGC revenues by 10% and is part of the attempt to rationalise Britain's energy industries.

The development came after news that additional coal resources amounting to more than 200 million tonnes have been discovered in Warwickshire. The seams compare with the Selby and Vale of Belvoir fields, and are the result of the National Coal Board's

exploration programme. A new £50 million three-year programme is due to start soon, and more discoveries are expected.

But not everything is rosy. Coal prices are also going up next month; NCB projections of output until the 1980s under its Plan for Coal show that exploitation of new mines is beset by problems in obtaining planning approval; and productivity shows a downward trend.

On the oil front the important matter of depletion policy in the North Sea is attracting attention. The issue emerged clearly recently in the Department of Energy's broad review for the NEDC. An annex stated that large-scale licensing in the early 1970s was designed to build up an exportable surplus in the 1980s; delays in starting work on new fields and "slippage" in the build-up of commercial fields, however, had "somewhat eroded the level of production expected in the early 1980s and therefore the export surplus".

Last week a slightly more optimistic prognosis for the export surplus was produced by a policy adviser to the British Petroleum Board, who counselled against a strict depletion policy designed to prolong the period of self-sufficiency. The issue looks like becoming increasingly important as the time for critical nuclear power decisions draws closer.

Arguably the most important of those decisions concerns the fast breeder reactor. One report this week says there are suggestions for a supervisory board, representing all concerned interests, to manage the project; an executive team of experts would report to it. The Prototype Fast Reactor at Dounreay, due to be shut down recently for a ten-week overhaul and for re-fuelling, will meanwhile take over the role of the small experimental reactor, which will be closed in a ceremony next week after some 17 years of service. In the reprocessing field Mr Benn's intervention last weekend has seen the end of the six-week industrial dispute at the British Nuclear Fuels plant at Windscale. □

EARTHQUAKES in Romania have many unusual features to them, not least a somewhat erratic occurrence rate. The recent quake of magnitude greater than 7 near Ploesti, which has caused such widespread damage and the loss of over a thousand lives, seems to conform to the pattern.

There is regular seismic activity in a band running down the east coast of the Adriatic, through the Aegean Sea and off into Northern Turkey, but further north, activity is rare, suggesting that the major active plate boundaries run well south of Romania. There is, however, a little activity along the north coast of the Black Sea, and a small isolated pocket of Romanian quakes lies along the continuation of this line at the southern most limit of the Carpathian mountains. These quakes are at a depth of 50 to 150 km and lie in a vertical slab (*Nature* 228, 1176-8 (1970)); the slab is probably the relic of a small segment of ocean that was overridden millions of years ago but is still out of equilibrium with its surroundings. The earthquakes would then result from the release of stress as the slab settled.

Since the recent quake there have been several attempts to discern patterns in the times of occurrence of Romanian earthquakes. Rarely can credence be attached to such statements which have been made on the basis of a rapidly compiled historic compilation. Although the catalogues

back to about 1900 are quite good for large earthquakes, the estimate of magnitude for many of these events leaves a lot to be desired; any analysis which then looks at the statistics of

Quake patterns



BACKGROUNDER

events above a certain magnitude is bound to be using shaky premises.

Even so, the suggestion emerging from the US Geological Survey (and regrettably inflated into an ambassadorial message) is of interest. It has been noticed that six of the eight quakes since 1900 which the Survey regard as large have occurred in

pairs, separated by a month or so. This is quite an unusual observation for earthquakes, and the prospect of a second large quake cannot be discounted. Prediction techniques based on rock dilatation that are beginning to emerge as important for shallow quakes seem difficult if not impossible to apply at present to events as deep as the Romanian quakes.

The earthquake was felt over a very wide area—Moscow, a thousand miles away, was shaken slightly. This had been noted for previous shocks and probably arises from a combination of two factors. First, an earthquake at depth, away from the near surface inhomogeneities, irradiates a larger area than does a shallow shock. And second, there is probably very little absorption of elastic waves crossing an old, stable region such as Western Russia.

Legal proceedings, it is reported, are to be brought against architects whose modern buildings collapsed in the quake. There is a certain amount of sense in this. An earthquake of comparable dimensions occurred in 1940 and was destructive; it seems that in some cases appropriate lessons have not been learnt. Earthquake-proofing of buildings is by no means an impossibly expensive task, and is done routinely in California and Japan, with great success. Maybe one of the good things to emerge from the disaster will be a greater insistence on building to safer standards.

IN BRIEF

Science budget breakdown

The Department of Education and Science (DES) was unable to comment this week on a report in *THES* detailing the breakdown of the Science Budget for 1977-78. The figures have yet to be agreed formally with the Treasury and passed by the House of Commons.

The Science Research Council's budget is believed to break down as follows: nuclear physics, including the CERN subscription, £43.3 million; astronomy, space and radio, £27.6 million; science, £23.3 million; engineering, £14.5 million. Figures for the other research councils are reported to be as follows: MRC, £42.3 million; NERC, £29.5 million; ARC, £20.4 million; SSRC, £14.5 million. The size of the overall budget was foreshadowed in the recent expenditure White Paper.

NASA change

James C. Fletcher, who has been head of the National Aeronautics and Space Administration (NASA) for the past six years, is being replaced, another casualty of the Carter Administration. He announced last week that he will leave on 1 May. The post is a Presidential appointment and Fletcher consequently submitted a pro-forma resignation to President Carter along with other holdovers from the Ford Administration, but it was no secret that he wanted to be kept on. No reason has been given for accepting his resignation and no replacement has been announced.

UN conference opens

Experts from some 150 countries were Argentina, this week for the opening

of the two-week UN Water Conference. The major item for discussion highlighted last year's Habitat conference in Vancouver: providing clean and safe water supplies to residents in urban and rural areas as soon as possible. Also on the agenda: pollution control, conservation of water resources, management of shared lake and river resources, water as a disease carrier.

Psychiatrist's death

The death has been recorded of Professor Daniil Lunts, head of the fourth section (political) of the Serbskii Institute of Forensic Psychiatry.

Last weekend a young paramedic, Aleksandr Podrabeinnik, who has collected details of over 200 cases of sane mental hospital internees in the Soviet Union, had his flat searched and papers confiscated.

THE debate on recombinant DNA is so far a tribute to the power of the human imagination rather than an acknowledgment of actual results obtained in laboratories. Most of the stronger statements seem to be made by people who are not trained in biology. However, some good ammunition has been provided by prominent scientists. For example, Robert Sinsheimer recently advanced the possibility that DNA from *Clostridium botulinum* might be used by a 'terrorist group' to transform *E. coli* into producing botulinus toxin. Obviously, such a group will not apply for a permit from the National Institutes of Health.

The environmentalist societies have reacted promptly and swiftly to the issue. The decision was easy, because in matters of science and technology, especially new ones, they are guided by the principle stated by Groucho Marx in the 1930s:

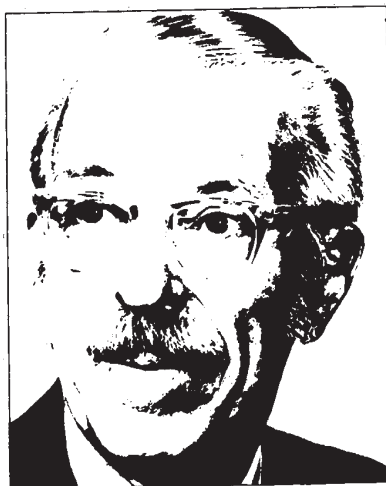
I don't know what you're going to say
It makes no difference anyway
Whatever it is, I'm against it!

Some of the busiest recombinant DNA laboratories are in our own digestive tracts, where the transducing phages, episomes and Hfr strains have been switching DNA around ever since the days of early evolutionary history. Knowledge of this should (but won't) have a calming effect on those who believe that recombination is a recent invention of maniacal molecular biologists.

I am impressed above all by the fact that nature buries her countless numbers of mistakes. The two-headed calf does not survive. Nor does nature help us in our genetic manipulations.

The most productive strains of our major crop plants would rapidly vanish without human protection and nurture. Maize would be the first to

Monster debate



THOMAS H. JUKES

disappear. It is true that there are deadly pathogenic micro-organisms in our midst but the number of species of these is comparatively small. They are the product of millions of years of commensal evolution with their hosts. The thought that we could slap new ones together in the lab and that they would be viable enough to run riot seems faintly ridiculous.

Even such an apparently obvious advantage as antibiotic resistance does not easily persist. Some years ago I participated in launching an experi-

ment that spread widely; the addition of antibiotics to the feed of domestic livestock. Although this has recently been criticised and scrutinised, the strange fact remains that it 'still works' when by all predictions the spread of resistant strains should long ago have made the procedure useless. This is especially so because resistance in enteric bacteria can spread by DNA recombination. I presume the wild, susceptible strains must tend to overgrow the resistant ones.

Public understanding of recombinant DNA is greatly aided by previous conditioning to science fiction. A long-time staple subject for cartoonists in popular magazines is typified by a picture showing 'mad scientists' in a laboratory where enormous rats are emerging from a flask, captioned 'Something Went Wrong'. This theme, almost to the letter, was used in a recent cartoon, and, to make the point absolutely clear, the scientists wore lab-coats labelled 'MIT'.

So far, the one predictable result of 'recombinant DNA research,' even the research that hasn't been done, is a bureaucratic explosion of paperwork. Any mention of DNA in a research grant proposal presses the alarm button, and a large package of questionnaires arrives in the mail. We can probably look forward to a new National Institute for Regulating Recombination, and even to its counterparts in state and local governments. Also, the term 'recombinant DNA' rolls trippingly from the tongue, and politicians know a vote-getting issue when they see one.

correspondence

The toxicity of plutonium

SIR,—We wish to comment on three recent publications, all originating in the United Kingdom, which have discussed the radiotoxicity of inhaled, insoluble plutonium compounds. In a recent article in *Nature*, Thorne and Vennart¹ concluded that the previously accepted value for the maximum permissible annual intake (MPAI) of such compounds may be too high by a factor of about five. A similar conclusion had previously been reached in a Medical Research Council report², and in a report³ by the Royal Commission on Environmental Pollution. Our main purpose here is to point out:

- that the proposed changes in the MPAI and in the closely-related maximum permissible air concentration (mpc) do not necessarily imply any change in the present procedures for assessing doses and risks in situations where persons have known amounts of insoluble plutonium in their lungs;
- that although the article in *Nature* and the MRC report come to broadly the same final conclusions, the reasons for the conclusions are significantly different; and that the uncertainties in the calculations, to which attention is drawn in both papers, are as large as the proposed reduction factor;
- that the observed behaviour of the technologically-important high fired plutonium oxide in people who have accidentally inhaled such material is significantly different from the behaviour predicted from the lung model assumed in recent MPAI calculations.

Maximum permissible annual intake. Thorne and Vennart stress that the MPAI which they derive is intended for planning purposes¹. We believe that most facilities in which plutonium oxide is handled are planned on the basis that there will be virtually no chronic exposure, and that this is also true in operational practice; hence the precise value of the MPAI (or the mpc) is somewhat irrelevant. However, exposures do occur from time to time, due to unplanned but identified incidents which usually cause the exposure of one or two individuals to quite high concentrations for a short time. In such situations, we believe that it is good practice to make the best estimates of intake, dose and risk to those specific individuals on the basis of measurements taken on them, with no reference to the concepts of MPAI (or mpc). In fact, such a procedure is

explicitly recommended⁴ in *ICRP Publication 19* on the page following the one on which they describe the model used in *Nature* and the MRC report.

Uncertainties in the MPAI. Although the *Nature* article and the MRC report both conclude that a reduction in the MPAI of the order of a factor of five is to be recommended, the two sets of calculations show significant differences. For example, the respective estimates of risk of bone cancer differ by more than a factor of five and the risks of lung cancer by more than a factor of three. On reading the respective texts it is obvious where the differences lie (in the relative importance of the respiratory lymph nodes, in the quality factor for alpha particles and in the calculations of dose to the bone). Both publications acknowledge that the estimates of 'nominal risk' are open to criticism and that the data on which they are based can be interpreted in several ways. It is an unfortunate fact that such qualifications are often disregarded by those who draw conclusions from the final tabulated presentation.

The maximum permissible lung burden (MPLB). In the summary of conclusions (paragraph 526.2) of its sixth report, the Royal Commission on Environmental Pollution³ claimed that a reduction by a factor of five in the MPLB is already practised. This statement appears to have resulted from a misunderstanding; we believe that their statement was intended to apply to the MPAI. The conclusion stated in paragraph 526.2 does not follow from the section of the report in which the matter is discussed (i.e. paragraph 77). Neither the MRC report nor the *Nature* article explicitly suggested a reduction in the MPLB, and in our view, no such reduction is required in the case of high fired plutonium oxide.

ICRP lung model. As explained above, all of the recent calculations have been based on the use of the lung model of *ICRP Publication 19*. The parameters given in the model were derived mainly from animal data, and presumably represent at best the mean or median values of a number of widely differing experimental values. Consequently, as explained in the associated text of the *ICRP publication*, the model can only be used for deriving general exposure limits; to evaluate a specific exposure situation one should choose the data most relevant to that particular situa-

tion. Measurements made on a substantial number of individuals^{5,6}, who had inhaled high fired plutonium oxide, show that the material initially deposited in the lung is removed much more rapidly than the *ICRP model* would suggest for what it calls a Class Y material. Moreover, experience here at Winfrith (to be published) has shown that the amount of material transferred from the lung to other parts of the body is also significantly less than the model would suggest; perhaps this is a consequence of the more rapid removal to which we have referred. As a consequence of these two factors, if risk estimates are made for those people who have inhaled high fired plutonium oxide, they are found to be a factor of about 5 lower than the risk estimates based on the use of the lung model, i.e. they are no higher than was previously supposed.

Discussion. We feel that the methods which have been used to derive new values for the MPAI are unduly pessimistic for high-fired plutonium oxide. That fact in itself does not worry us unduly. What does worry us is that people may be tempted to conclude, by the use of the same lung model, that in every case in which a person inhales insoluble plutonium, the risks to bone and liver are comparable to those of lung; and hence to conclude that the MPLB should be reduced. We believe that in the case of high fired plutonium oxide, the previously established idea that the lung is the critical organ remains substantially correct.

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¹ Thorne, M. C., and Vennart, J. *Nature* **263**, 555–558, 1976.

² *The Toxicity of Plutonium* (HMSO, London, 1975).

³ *Sixth Report of the Royal Commission on Environmental Pollution* (Chairman Sir Brian Flowers, Cmnd. 6618, HMSO, London, 1976).

⁴ *ICRP Publication 19: The Metabolism of Compounds of Plutonium and the Other Actinides* (Pergamon, Oxford, 1972).

⁵ Ramsden, D. *Diagnosis and Treatment of Incorporated Radionuclides*, 139–161 (International Atomic Energy Agency, Vienna, 1976).

⁶ Watts, L. *Health Physics* **29**, 53–59, 1975.

Registering Russian mail

SIR—Apropos of your article "Keeping in touch with Soviet colleagues" (10 February, page 484), it is my experience that correspondence with them is expedited by using registered mail.

P. A. DAVENPORT

Culham Laboratory, UK

news and views

Marburg and Ebola: viruses in search of a relation

from David I. H. Simpson and Arie J. Zuckerman

In the second half of 1976 two severe epidemics of haemorrhagic fever broke out in Sudan and Zaire. The virus responsible, provisionally named Ebola virus, proved to be structurally similar but antigenically different from that of Marburg disease, and three recent reports in *The Lancet* give details of its isolation and preliminary characterisation.

Marburg virus

Marburg virus disease, popularly but incorrectly named 'green monkey' disease, is a severe distinctive haemorrhagic febrile illness of man, first described in 1967 when thirty-one cases of illness with seven deaths in Germany and Yugoslavia were traced to direct contact with blood, organs or tissue cell cultures from a batch of African green monkeys (*Cercopithecus aethiops*), which had been trapped in Uganda. Several secondary cases occurred in hospital personnel by contact with the blood of patients, and one further case was apparently transmitted by sexual intercourse 83 days after the initial illness. The case fatality rate was 29% for the primary cases, but there were no deaths in the six secondary cases. It soon became apparent that this was a previously unrecognised disease caused by an infectious agent probably new to medical science.

The possible origin of the infection has been investigated since the 1967 outbreak. Complement fixing antibodies to the Marburg virus were reported from a laboratory in the USA in many African monkeys, but conflicting results were obtained in other surveys and it appeared that the antigen used in the USA was not entirely specific. Furthermore, before 1967 many thousands of monkeys had been handled in biomedical laboratories without such outbreaks, and in addition

experimental infection of monkeys proved uniformly fatal. The natural cycle of transmission of the virus in various species of animals and the origin of the infection are still under intensive investigation.

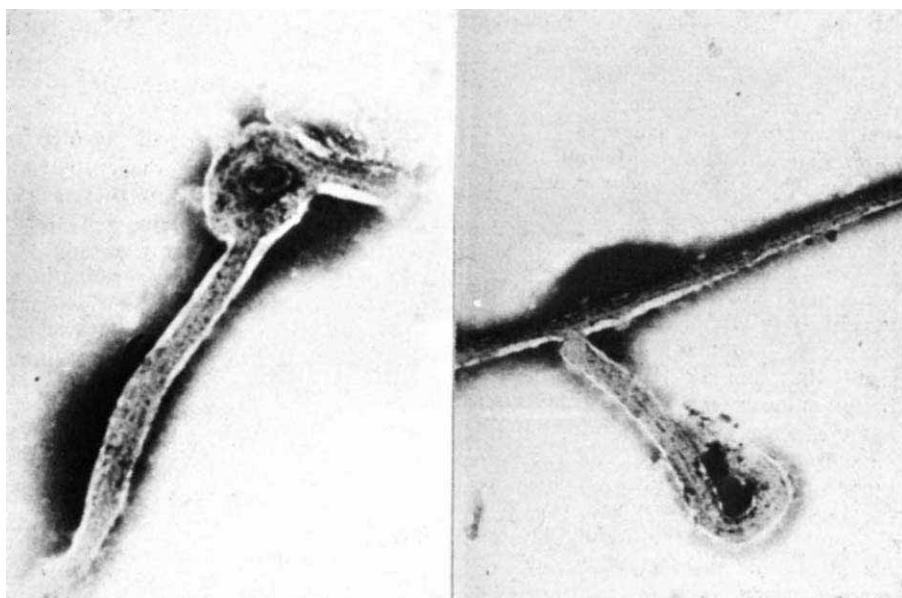
The first recognised outbreak of the disease in Africa and the first since the original outbreak, occurred in South Africa in February, 1975. The primary case was a young Australian man who had hitch-hiked through Rhodesia. He died in a Johannesburg hospital and shortly afterwards his girl travelling companion and one of the nurses who had cared for him fell ill with the same disease. The girls recovered. Virological studies showed that this outbreak was caused by the Marburg virus.

As in the original Marburg outbreak, when virus was isolated from seminal fluid 83 days after the onset of illness,

evidence was obtained that the virus can persist in the body for at least 2-9 months after the initial infection. In the Johannesburg episode, virus was cultured after infection of the eye from fluid aspirated from the anterior chamber of the eye 80 days after the onset of the illness (Gear *et al. Brit. med. J.* 4, 489; 1975).

Ebola virus

Three recently published papers by Pattyn, Bowen and Johnson and their colleagues (*Lancet* i, 569-574; 1977) now give details of the isolation and preliminary characterisation of the virus responsible for two severe and almost simultaneous epidemic outbreaks of haemorrhagic fever in the equatorial provinces of Sudan and Zaire in the second half of 1976. The isolates were found to be structurally indistinguishable from strains of Marburg virus



Electron micrograph of the Zaire prototype strain of Ebola virus (courtesy of E. T. W. Bowen and W. J. Harris).

isolated in Germany in 1967 (Siegert *et al. Dtsch. med. Wschr.* 92, 2341; 1967) and South Africa in 1975, but antigenically the Sudanese and Zaire isolates although identical to each other were quite distinct from Marburg. The prototype of these new strains has been named Ebola, after a small river in Zaire which flows north of Yambuku, the village of origin of the patient from whom the first isolate of the virus was obtained. Experimental studies in laboratory animals (E. T. W. Bowen, unpublished results) confirm the antigenic differences since previous immunity to Marburg virus does not protect against subsequent infection with Ebola virus and *vice versa*.

Morphology

Marburg and Ebola viruses show no antigenic relationship to any other animal virus and morphologically they belong to no known virus group. Marburg virus contains RNA, has an outer envelope containing lipid, and is ether sensitive. Both viruses are formed by budding through cell membranes and it has been suggested (Siegert *et al. op. cit.*) that Marburg resembles members of the rhabdovirus group. However, the size of the central core and the cross-sectional structure differs from that of rhabdoviruses.

Two principal structural forms of Marburg virus have been described (Almeida *et al. Marburg Virus Disease* (eds Martini & Siegert) 85, Springer-Verlag, Berlin, 1971): an elongated filamentous or sinuous form, which could be described as tubular; and a circular form in the shape of a 'doughnut' or torus.

Both forms have an overall diameter of 70–100 nm excluding projections on the outer surface and contain an internal helix 40 nm in diameter. Several of the sinuous particles are extremely long, often several microns. Almeida and her colleagues suggested that as mature virus particles give rise to circular torus forms Marburg might be regarded as the founder member of a group known as the Toroviruses. However the predominant forms of Marburg and Ebola most frequently seen in the electron microscope in negatively stained preparations (Figure) are long tubular structures and since they contain RNA perhaps a better name might be Tubonaviruses, a name suggested at an informal discussion between K. M. Johnson and ourselves. Geographical names for diseases or their causes are also to be discouraged since these may be misleading, implying as they do an unusual association with a particular country or town, and may then lend a sinister connotation to the area, as

in the case of Lassa, a town in North-Eastern Nigeria and Marburg, a University town in Germany.

Epidemiology

It has now been generally accepted that monkeys are not the natural reservoirs of Marburg infection. Intensive epidemiological investigations in Southern Africa following the three cases in Johannesburg in 1975 failed to provide many clues to the natural maintenance host of the virus. Similar investigations in Sudan and Zaire have not yet uncovered the reservoir of Ebola virus. The Zaire and Sudan outbreaks involved well over 500 cases and at least 350 deaths. The illness was marked by a sudden onset of severe headache, fever, muscle pains and prostration, quickly followed by profuse diarrhoea and vomiting and later in many severe cases by dramatic haemorrhages, and closely resembled the illness described in Germany in 1967 and in South Africa in 1975. The typical skin rash so common in white-skinned patients was not so distinct in black

skins. In each outbreak, be it Marburg or Ebola fever, attendant medical and/or nursing staff became infected often with disastrous results. Two hospitals in Zaire and Sudan were brought to a standstill; in one hospital in Sudan 76 staff became ill and 41 of them died. Direct human-to-human transmission was responsible for all the secondary infections usually brought about through close and prolonged contact with sick patients and particularly through contact with patients' blood.

The fact that Marburg and Ebola viruses once they have been transmitted to man through this contact with an unknown reservoir host can adapt themselves to direct man-to-man transmission means that these severe infections could easily be introduced from their country of origin to countries where the natural host does not exist. Hence the international concern over these infections and others such as Lassa fever. It is therefore all the more important that detailed epidemiological investigations are carried out without delay. □

Trypanosome antigens

from F. E. G. Cox

INFECTIONS with African trypanosomes are long-lived and are characterised by waves of parasitaemia in which the parasites seen in the blood at any one time differ antigenically from those of preceding waves. This antigenic variation is thought to be responsible for the prolonged infections in what ought to be immune hosts because, although the hosts make antibody to each antigenic variant, they are foiled when the antigens of the trypanosomes change and each becomes effectively another parasite. The mechanisms of antigenic variations are interesting from both theoretical and practical viewpoints and over the past few years three important questions have been asked. First, are variant antigens situated in the surface coat, second, if so, are they produced by the trypanosomes or are they accretions of host serum proteins and, third, do all trypanosomes carry a range of variant antigens or do certain individuals possess one kind of antigen and others a different kind? In the case of *Trypanosoma brucei* these questions have now been satisfactorily answered.

It is now known that the variant antigen actually constitutes the majority of the surface coat of the trypanosome (Cross *Parasitology* 71, 393; 1975; Cross & Johnson *Biochemistry of Parasites and Host Parasite Relationships* (ed. van den Bossche, H.) 413; 1976) and that it is a glycoprotein consisting of a single polypeptide chain of molecular

weight 65,000. The different variants are not simply minor modifications of a basic type because when the amino acid sequences of related variants are analysed they are found to be completely different from one another (Bridgen, Cross & Bridgen *Nature* 263, 613; 1976). So the answer to the first question is that not only are the variant antigens associated with the surface coat, they actually form much of it and furthermore the successive variants represent structurally dissimilar polypeptide chains. Could these have come from the host serum? A recent paper by Taylor and Cross (*Parasitology* 74, 47; 1977) proves that they do not. When trypanosomes are maintained *in vitro* in the absence of host material variant antigens are still synthesised and incorporated into the surface coat. This confirms the original hypothesis of Vickerman (*J. Cell Sci.* 5, 163; 1969) and refutes the suggestion by Desowitz (*Immunity to Parasitic Animals* 2, (eds Jackson, G. J. *et al.*) 551; 1970) that the variant antigen is assimilated from the host.

Trypanosomes revert to a basic antigenic type after they pass through the tsetse-fly vector and the subsequent variants appear in a recognisable sequence. This occurs even in infections initiated with single parasites (clones) and this has been interpreted as meaning that trypanosomes do not exist as mixed populations with different individuals expressing different variants but

actually continually change their antigens. However, experiments by van Miervenne *et al.* (*Annl Soc. belge. Med. trop* **55**, 1, 55, 25; 1976), using a fluorescent antibody technique to identify particular variants, have shown that clones rapidly become antigenically heterogeneous and that in every population there are a few individuals that do not bear the antigen characteristic of the population. These individuals are at an advantage when the immune response to the predominant antigen begins to become effective but are in their turn destroyed as they become dominant and have to give way to yet other variants.

Cross and his coworkers suggest that trypanosomes express a gene for and synthesise a single variant antigen at a time. Over evolutionary time a number of genes each coding for a particular variant have emerged by mutation, been selected for and have been accumulated giving the trypanosomes of the present time a vast repertoire of variants which they need to survive in their mammalian hosts. None of the available evidence contradicts this hypothesis and the next questions must be how the antigen switch is brought about and, when this is known, how it can be stopped. To those living and trying to raise cattle in the tsetse belt of Africa this is not just an academic question. □

Prenatal detection of thalassaemia

from Sue Malcolm

THE various inherited haemoglobin disorders which collectively go under the name of thalassaemia are the most serious genetic diseases in many parts of the world. So the successful prenatal diagnosis of both the α and β thalassaemias marks a significant advance in their prevention.

α thalassaemia (in which no α globin chains are produced) is now known to be caused by the deletion of the DNA coding for the α chain (Ottolenghi *et al.* *Nature* **251**, 389; 1974; Taylor *et al.* *Nature* **251**, 392; 1974), and Y. W. Kan and colleagues in San Francisco have now devised a method of determining whether the α globin genes are present in foetal DNA (*New Engl. J. Med.* **295**, 1165; 1976).

The foetal DNA was extracted from cultured amniotic fluid cells obtained by amniocentesis during the fifteenth week of pregnancy and cultured for a further five weeks to provide enough DNA for analysis. Radioactively-labelled α globin complementary DNA synthesised on α globin mRNA was

then hybridised to the foetal DNA. The results from this technique are quite clear cut but require the greatest precision in mixing the two DNAs and care in making the measurements.

At the ratios of cDNA to DNA used there should be three α globin minus strands (two from the cold foetal DNA and one from the cDNA) to every two plus strands in normal DNA, and therefore the radioactive probe should be 66% hybridised. If two α globin genes are deleted (α thal I) the ratios would be two minus strands to one plus strand, and only 50% hybridisation found, and if three genes are deleted (Hb H disease) the minus:plus ratio would be 3:1 and 33% hybridisation found. If in fact all four α genes are deleted in the foetus (hydrops fetalis) no hybridisation would be expected. Unfortunately the α globin cDNA was only 80% pure, the remaining sequences being β cDNA so even in a case of hydrops fetalis around 20% hybridisation would be found. A successful diagnosis therefore rests on the ability to distinguish between 20% and around 45% hybridisation (~125 c.p.m.) since only the difference between hydrops fetalis and Hb H is clinically important. In the case reported Kan successfully predicted that the foetus examined had α thalassaemia I. Later, twins were diagnosed and the parents had no hesitation in continuing the pregnancy since at least one foetus was known to be unaffected. In the event healthy twins, one male and one female, were born. At least some of the uncertainty in this type of diagnosis will be removed when cloned, and therefore pure, human globin sequences become available.

An even more serious health problem is posed by the diseases associated with faulty β globin chain production, β thalassaemia and sickle-cell anaemia. Reports from a combined Boston and London study (Alter *et al.* *New Engl. J. Med.* **295**, 1437; 1976) and from Kan's San Francisco group (*Lancet* **i**, 269; 1977) announce encouraging progress in the prenatal diagnosis of both these diseases. In both cases there is an error of β globin expression rather than a gene deletion; in β thalassaemia no β globin chain is produced and in sickle-cell disease a mutant globin chain (β_s). Therefore analysis can only be carried out on cells which actually produce globin—the foetal blood cells.

The biochemical problem has been elegantly solved. The blood cells are incubated with ^3H -leucine and the resultant globin chains analysed on carboxymethylcellulose chromatography columns. In the case of β thalassaemia the β/γ globin ratio is measured and in the case of sickle-cell disease the presence of β_A or β_s is

determined. Prenatal diagnosis is possible because even in the first trimester of pregnancy 10% of the β -like chains produced are β rather than the foetal γ globin.

Serious problems in interpretation could arise if the blood sample were contaminated with maternal cells. The Boston-London group overcame this problem by transfusing the mother to reduce her reticulocyte (globin-producing cell) count before foetal blood sampling and the San Francisco group used anti-i serum, an antibody that agglutinates foetal cells. Both groups detected homozygous β thalassaemia and homozygous sickle-cell disease *in utero* and their results were confirmed after abortion.

However, both groups also found foetal blood sampling a hazardous procedure and altogether 6 out of 55 fetuses were lost as a result of attempting to sample foetal blood (three from each centre). The Boston-London group used placental aspiration when there was an anterior placenta and foetoscopy for a posterior placenta. The San Francisco workers used placental aspiration in both cases. The Boston-London workers found foetoscopy less hazardous and since their very first attempts they have had far greater success. The one case in which they wrongly predicted a healthy baby was the result of an insufficient foetal blood sample.

Although the results of the two groups combined are very encouraging it is clear that attempts to take foetal blood during early stages of pregnancy can be very dangerous and the continuing use of these methods for prenatal diagnosis depends on this procedure's becoming much safer. As pointed out in an editorial in the *British Medical Journal* (**531**, 532; 1977) it will also be necessary to simplify the biochemical procedures and the apparatus involved before these techniques can be applied in the parts of the world where these diseases are common. □

Polarity time scale extended by 2 Myr

from Peter J. Smith

WITH the possible exception of any very short events remaining undetected, the details of the geomagnetic polarity time scale based on radiometrically-dated subaerial igneous rocks of the past 4.5 Myr have been known for some time. It would now clearly be desirable to extend this well-established

scale backwards, partly to increase its usefulness as an aid to stratigraphic correlation and partly to reduce the errors involved in matching it to much longer polarity reversal patterns (for example, from oceanic magnetic anomalies and deep-sea sediment cores) based largely on relative dating. Unfortunately, however, this is far from easy. For one thing, with increasing age it becomes more and more difficult to find rocks which remain unaltered and thus not subject to geological 'errors' such as loss of radiogenic argon. But even where suitable material is available, beyond 4–5 Myr, potassium–argon ages have precision errors comparable to many of the time intervals to be resolved.

One way out of the latter dilemma, as McDougall *et al.* (*Geol. Soc. Amer. Bull.* **88**, 1; 1977) have found, is to concentrate on a long stratigraphically controlled sequence of rocks and rely on mathematical techniques to smooth out data points which individually would not be sufficiently precise to define polarity boundaries. The sequence that McDougall and his colleagues chose comprised more than 400 successive lavas in Borarfjörður, western Iceland, with a total thickness (including interbedded sediments) of more than 3,500 m. The palaeomagnetism was straightforward; most of the lavas gave reliable directions and thus polarities and a reversal pattern. The success rate for potassium–argon analysis, on the other hand, was much lower. In fact, only 24 samples were considered suitable for dating on the basis of minimal alteration of primary mineralogical phases and minimal development of secondary minerals.

The selection of rocks for radiometric dating is admittedly still largely subjective. Although fresh well-crystallised lavas are generally suitable and highly altered lavas are obviously useless, there is particular difficulty in deciding whether or not only slightly altered samples should be rejected, especially where (as in Iceland) completely unaltered rocks are so rare that little progress could be made with them alone. In this case, however, there were ways of assessing the overall reliability of the results obtained. For example, the 24 potassium–argon ages determined by McDougall *et al.* were generally consistent with the stratigraphy, suggesting them to be fairly good indicators of the ages of crystallisation of the lavas rather than of the age of any subsequent burial metamorphism. Indeed, they showed no correlation at all with the positions of the rocks with respect to Iceland's well-mapped zeolite zones.

So accepting that their measured ages were "valid estimates, to a first

approximation, of the time since the lavas were erupted", McDougall and his coworkers then proceeded to carry out a simple linear regression analysis of determined age against the aggregate thickness of the lava–sediment sequence measured from its base. Notwithstanding the implicit assumption here that the sequence accumulated at a uniform rate throughout, the best-fitting age–thickness curve gave an age of 3.27 Myr for the clearly-recognisable Gauss–Gilbert boundary. This is in good agreement with the well-established age of 3.32 Myr for this boundary, suggesting that both the basic data and the assumptions made in analysing them were valid. Even so, to normalise the data a new regression analysis was carried out using only rocks older than the Gauss–Gilbert transition fixed at 3.32 Myr.

The revised regression line together with the polarity data give an age of 5.34 Myr for the Gilbert–Epoch 5 boundary, 5.83 Myr for the Epoch 5–Epoch 6 boundary and 6.54 Myr for the Epoch 6–Epoch 7 boundary. The Cochiti and Nunivak normal events within the Gilbert reversed epoch appear as a single event lasting from 4.09 to 3.86 Myr ago—a failure of resolution possibly arising from a hiatus in the eruption of lavas at the critical time. However, the two normal events in the lower Gilbert, termed C_1 and C_2 by Opdyke (*Rev. Geophys. Space Phys.* **10**, 213; 1972), appear separately, covering the periods 4.45–4.33 Myr and 4.81–4.60 Myr, respectively. The names 'Sidufjall event' for C_1 and 'Thvera event' for C_2 are now proposed.

As for older epochs, the unnamed reversed event previously recognised within normal Epoch 5 by several groups of workers is now seen to cover the period 5.50–5.37 Myr whereas the unnamed normal event previously seen within reversed Epoch 6 covers the period 6.39–6.27 Myr. But McDougall *et al.* also find two further normal events within Epoch 6—very short ones at 6.43 Myr and 6.12 Myr. Apparently these have not been detected previously either in marine magnetic anomalies or in deep-sea cores.

No doubt some of these ages (which have uncertainties of 0.1–0.2 Myr) will be subject to amendment by subsequent workers; it would be surprising if that were not to be so. In the meantime, however, McDougall and his colleagues have made a major contribution in extending the polarity time scale based on subaerial rocks back about 2 Myr further than previous attempts. Incidentally, the extended scale now covers the Miocene–Pliocene boundary for which McDougall *et al.* propose a revised

age estimate of 5.2 ± 0.1 Myr although admitting that there may be data (for example, from New Zealand) inconsistent with this figure. □

Earthquake prediction

from E. R. Lapwood

The Fifth US–Japan Seminar on Earthquake Precursors, sponsored by the Japan Society for Promotion of Science and the National Science Foundation, met in Tokyo on 17–19 January 1977. It was organised by Professors Z. Suzuki and C. Kisslinger and the proceedings will be published as a special number of the *Journal of The Physics of the Earth*.

HOPES that monitoring of the ratio of speeds of sound and shear waves in stressed rock would be the key to earthquake prediction have been disappointed, but Japanese and American seismologists believe that successful prediction will be achieved. When, however, will depend on the intensity of effort, on the level of funding and on effective international cooperation.

In both Japan and the US the budget directly provided for earthquake prediction research runs into millions of US dollars. (The 1976 US programme was for \$5 million, and the Japanese—much greater—for \$8 million not including salaries. Seismologists in both countries are hoping for increased support in 1977.) Especially in Japan this earmarked budget is to be seen against a background of highly efficient routine observations and widespread research in universities. Papers presented to the seminar showed that understanding of earthquake origins and mechanisms is advancing on a wide front; and that that is essential in view of the complexity of the problems and the variety of possible premonitors.

Experimental work on the behaviour of rocks (and other materials) was described by W. Brace (Massachusetts Institute of Technology) and K. Mogi (Tokyo University). Brace emphasised the important differences in behaviour of intact rock and 'gouge' (the crushed material created at the rough interface by sliding on a fault), in particular as regards dilatancy (expansion just before breakdown) and its accompanying effects. At a huge fault with a history of repeated movements in recent time the layer of gouge may be very thick, whereas at a fault with no such character it may be thin; thus the response of the material just before slip on the fault, and premonitory signals, may be

very different. Mogi described his experiments on dilatancy of rocks under triaxial stress. Previous experimental work had been mainly with axial symmetry. When the three principal stresses are unequal the response is more complicated and more realistic, so that Mogi was able to describe effects very relevant to earthquake mechanics.

The importance of Japanese records often emerged. For instance W. Thatcher (US Geological Survey) reported that analysis of peat layers in a trench cut across the San Andreas fault enabled movements in the past 1,500 years to be listed, giving an average interval of 170 years between large local movements. But T. Matsuda (Tokyo University), using Japanese historical records, was able to classify all active (that is, Quaternary) faults in Japan according to estimated slip rate and recurrence time, and to set upper and lower bounds on the greatest earthquake to be expected on a given fault at a given time. Again, the recent discovery of anomalous uplift between 1960 and 1974 in Southern California has led US agencies to allocate \$2.1 million for its observation and study. In Japan, on the other hand, 20,000 km of levelling has been covered five times since 1884 (except for Hokkaido) and geodesists are already in a position to detect anomalous uplifts in any part of the country. At present the Tokai region (southwest of Tokyo) has been designated as an "area of intensified observation", partly because of increased crustal movement since 1974.

E. R. Engdahl (NOAA) described the results obtained by himself and C. Kisslinger (University of Colorado) from two high-gain high-frequency seismograph networks in the central Aleutians. The high level of seismic activity and the precision with which epicentres within the net, and depths of foci, could be located produced splendid maps and cross sections, by means of which the migration of activity could be followed, and the pat-

tern of foreshocks and aftershocks of a moderate ($m_b 5$) earthquake examined in remarkable detail. Equally impressive were the results achieved at the Observation Centre for Earthquake Prediction at Tohoku University. A. Takagi (Tohoku) showed graphs of the hypocentre (focus) distribution of intermediate-depth micro-earthquakes below the North-East Japan arc. He demonstrated a double-plane structure, sources lying on parallel dipping planes separated by 30–40 km.

S. Suyehiro (Japan Meteorological Agency) described the close coverage of the Japanese islands with seismograph stations, and plans for telemetering and for emplacing ocean-bottom seismographs which in a few years should enable the hypocentres of earthquakes of magnitude $M > 3$ to be computed in real time (as the earthquakes happen). Fault-plane solutions, describing the source mechanism, are routinely computed for events of $M > 5$. Local seismograph networks, operated by various universities, are able to see the fine structure of hypocentres among foreshocks, aftershocks and seismic swarms.

But neither these agencies nor any other in Japan or the US has been able to identify regularly occurring seismic precursors in the form of anomalous velocity ratios or P delays. Only for compressive stress in hard rock—and not always then—do such precursors appear. Moreover, Japanese seismologists have a good deal of evidence that dilatancy is confined to crustal rocks. T. McEvilly (University of California, Berkeley), in a review of the present state of knowledge of precursors, gave the following rough estimates of thresholds above which a long-term coherent anomaly could be distinguished from noise (Table 1).

He considered that it was not yet possible to give a similar table for expected values of precursors; deformation precursors are well documented, but there is serious difficulty in pre-

dicting the time of the main event; other physical and chemical parameters have not yet been shown to give consistent indications; animal behaviour should, if it is significant, lead to measurable physical parameters.

Much interest was aroused by the paper by S. Suyehiro and H. Sekiya (Japan Meteorological Agency) on anomalous seismic activity (not classed as foreshocks) before seismic events, and by the paper by C. Scholz (Columbia University), on a possible deformation front moving across North China at about 110 km yr^{-1} at the time of the earthquake at Hai-cheng (Liao-ning province).

Having given a pessimistic estimate of the possibility of compiling during the next 10 years enough case studies of large earthquakes of different types to make prediction of destructive earthquakes feasible, F. Press (Massachusetts Institute of Technology) appealed for the pooling of resources in instruments and men for a cooperative effort from China, Japan, USA and USSR—the countries which now exert 90% of relevant worldwide research effort.

Z. Suzuki and C. Kisslinger (University of Colorado), joint organisers of the symposium, pointed to the rising public demand for earthquake prediction, and emphasised the need for seismologists to learn to speak intelligibly to ordinary people. H. Ohta (Tokyo University) and E. Haas (University of Colorado) reported surveys of social attitudes and responses; from these it was very clear that the education of the public to understand statements of probability and to respond rationally must precede announcements predicting earthquakes. In fact, it must begin at once in countries at seismic risk. □

Optical fibres at Williamsburg

from J. Baker

The Second Conference on Optical Fibre Transmission was held at Williamsburg on 22–24 February, 1977.

THE effort in fibre optics communications technology (the transmission of information by light pulses travelling down hair-like fibres of glass) is concentrating on realising on a practical level the inherent advantages of the system—high bandwidth, immunity from electrical interference, small size, and low cost.

Table 1

Differential magnetic field	1γ in 10 km
Electrical resistivity	1% change in 10 km
Electric field	$1 + \%$ change in 10 km
Atmospheric electric field	? 200%
Radon content in wells	20% in a few months
Water level in wells	Depends on local conditions
Strain	1 in 10^8
Tilt	1 microradian
Uplift	
Levelling	1 cm in 10 km
Gravity	$3 \times 10^{-8} g$
Differential sea-level	2–5 cm
Travel times of seismic waves	
Relative P delays	
(beyond 40° and between stations within 50 km)	0.1 s
V_p/V_s ratio	
(over periods of months, with good processing)	0.1
Velocity changes from local earthquakes	1%
Controlled sources	
(explosion, air-gun, vibrator)	10^{-4} – 10^{-3}

Many organisations reported the results of field tests on complete information-carrying networks suited to a variety of applications. N. Shimodaira (Nippon Electric Co.) reported the results of field tests on a high bit rate (400 Mb s^{-1}) optical link, the transmission of such high bit rates being made possible by two factors. First, the gradient of the refractive index in the fibre is carefully built up during fabrication, decreasing smoothly from the centre to a constant value in the "cladding". This results in nearly equal group velocities for the various propagating modes which each pulse of light excites. Second, the modes excited by the pulses of light are restricted to those of low order, thus further reducing the total spread. Ga-Al-As semiconductor laser diodes were used to launch pulses into the fibres, and avalanche photodiodes were used to detect them at the receiving end. At the other end of the spectrum W. H. Hackett (Bell Laboratories, Murray Hill) reported the results of tests on short-haul low bit-rate (16 and 32 Mb s^{-1}) data links incorporating light-emitting diodes to launch the pulses and PIN photodiodes to detect them.

Information transmission by way of optical links has inherent advantages in environments where high electric fields are present. T. Sekizawa (Fujitsu Laboratories Ltd) reported the results of a cooperative venture which has demonstrated this convincingly in field conditions. They found that the performance of their optical fibre links, which were routed through ducts containing power cables, were not measurably degraded by proximity to high voltages.

Several papers were devoted to splicing techniques. The contribution by P. K. Runge (Bell Telephone Laboratories, Holmdel) and W. C. Young (Western Electric) was notable, the connector described being moulded directly to the protective sheath of the optical fibre. The authors quoted losses per connector in the region of 0.4 dB. However it was clear that more work is necessary before a viable splicing system becomes available for general field use.

In the field of fibre fabrication a great deal of effort has been devoted to producing consistently very strong fibres. Both C. Kao (ITT Electro-Optical Products Division) and C. R. Kurkjian (Bell Laboratories, Murray Hill) quoted breaking strengths in excess of 500 k.p.s.i. for $125 \mu\text{m}$ diameter fibre by ensuring that surface defects and contamination of the fibres during manufacture were kept to a minimum. Fatigue strengths of fibres under applied stress were also reported

which indicated that potential service lives greater than 20 years can be expected for fibre now being produced.

The overall impression gained was that optical communications systems are now a viable proposition for capacity data links. Concerning the future for long haul telecommunications, the question is now how soon?

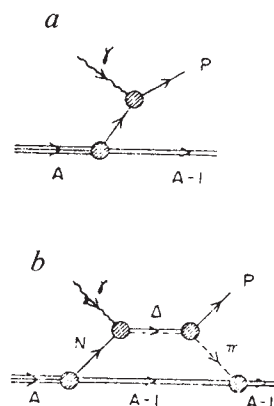
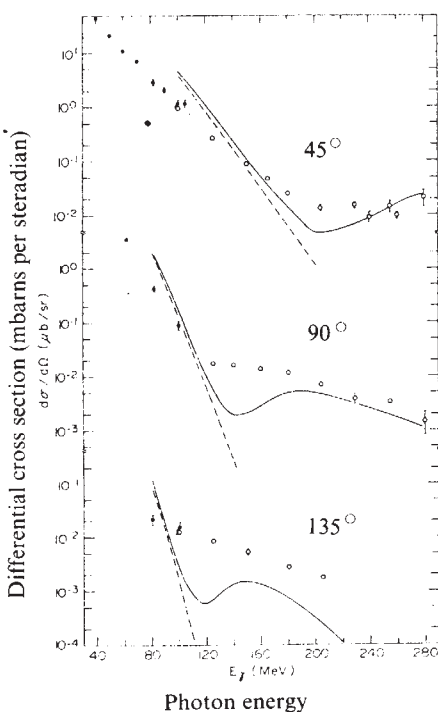
Short range nuclear forces

from P. E. Hodgson

EXPERIMENTS on the photo-proton reaction on oxygen from 100 to 300 MeV by a joint MIT-Glasgow group have recently provided evidence that in this energy range the reaction proceeds through the Δ (1232) nucleon isobar as an intermediate state, and it is likely that this process is increasingly important at high energies (*Phys. Rev. Lett.* **38**, 8; 1977).

The experiment was carried out by bombarding a beryllium target with a bremsstrahlung beam and measuring the spectrum of the emitted protons. The differential cross sections for the photo-proton reactions on oxygen that leave the residual nucleus in its ground state are shown for several angles as a function of photon energy in Fig. 1.

It is simplest to suppose that the reaction proceeds by a single-step process in which the incident photon interacts with a single proton in the target nucleus and knocks it out. This is indicated diagrammatically in Fig. 2a, and



gives the dashed curves in Fig. 1. It is clear that this theory, allowing for uncertainties in the calculations, is in qualitative accord with the data at low energies, but quite fails at higher energies. The calculations are rather rough, since harmonic oscillator wave functions were used, and the distortion of the wave function of the outgoing proton was neglected, but this does not affect the general conclusion.

The experimental cross sections at higher energies are much higher than those given by the one-step process. Such cross sections can be attributed phenomenologically to short-range correlations due to the repulsive core of the nucleon-nucleon interaction or a microscopic model can be made in terms of a two-step process. One such process, involving the Δ (1232) nucleon isobar excited in an intermediate state, is indicated in Fig. 2b. Calculation with this process gives the full curves in the figure, when added to the results of the calculations of the one-step process already considered. Although there are differences in detail due to the approximations made in the calculation, it is clear that the overall trends are qualitatively given. This provides evidence that the Δ -process can make a major contribution to the photo-proton cross-section in the 100–300 MeV region, and thus gives further insight into the structure of the short-range nuclear forces. □



A hundred years ago

THE Balloon Commission of the French Government, styling itself "Commission pour les Communications par Voie Aérienne," has become a standing institution, and includes within its province carrier pigeons as well as aeronauts and balloons.

From *Nature* **15**, March 15, 439; 1877.

review article

Some recent developments in remote sensing

P. Schagen*

In recent years electronic instruments have been developed which increase very considerably our ability to view the nocturnal scene at otherwise critically low incident light levels, by using the available reflected radiation more efficiently with the aid of image intensifiers. Still other instruments employ sensors which are sensitive in the infrared and convert the natural thermal radiation of any object in the scene into visible pictures. In this article recent technological developments in image intensification and thermal image conversion are briefly discussed.

REMOTE sensing depends on a large variety of techniques and finds application in a wide range of human interests. The techniques used may be 'passive' methods of obtaining information about a particular parameter of the environment, for example its local variations in emission or reflection of natural electromagnetic radiation. They may be 'active', using probing beams of light (as in laser radar systems and range finders) or irradiating the scene with X rays or ultrasound to obtain pictures for medical diagnosis or non-destructive testing.

It is impossible in a brief article to present a comprehensive survey of the field as a whole. Instead, the discussion has been restricted to some recent developments in two methods of passive sensing of the environment, using image intensification and thermal imaging. Both are of particular importance at very low levels of incident illumination.

Perception at very low light levels

In daytime the visual acuity of the eye is limited only by the retinal cone density and the optical aberrations of the eye lens^{1,2}, because the level of photon flux is far greater than the level of its statistical fluctuation. On the other hand, at night when incident light is reduced to starlight, airglow and occasionally moonlight, the numbers of photons captured by the eye are so small that their statistical fluctuations become the limiting factor. The eye will only perceive an object detail in the scene if it can detect the difference in illumination of two corresponding adjacent elementary areas on the retina. Each of these areas registers a number of photons, on average, during the integration time of the retina. The difference between these average numbers constitutes the photon 'signal', with an associated photon 'noise', caused by the statistical fluctuations in the numbers during successive integration periods. If the actual numbers of detected photons become so small that the photon-signal is indistinguishable from the photon-noise, then the eye is not able to sense the object detail.

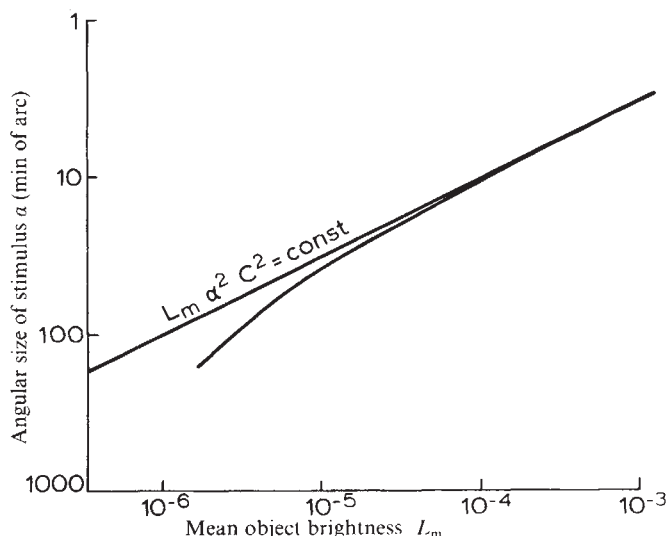
As light level decreases and photon-noise becomes an important factor, the eye tries to compensate for the reduction in the density of photons received in a number of well-known ways. First, the pupil dilates to let the eye capture more photons from the scene—the pupil diameter is 0.2 cm at a brightness of 10^4 cd m^{-2} , but rises to, and levels off at a diameter near 0.8 cm when the brightness is down to $10^{-4} \text{ cd m}^{-2}$. (10^4 cd m^{-2}

is roughly the brightness of white paper on a bright summer's day; $10^{-4} \text{ cd m}^{-2}$ is roughly the brightness of a cloudy moonless sky at night.)

Second, dark-adaptation takes place: the cones, the sensor elements of the retina which are active in daylight and are responsible for colour vision, cease functioning at a scene brightness below about $10^{-3} \text{ cd m}^{-2}$, and the rods, which are overloaded in daylight and which are not colour-sensitive, slowly recover and take over. An important aspect of this process seems to be the ability of the neurones associated with the rods to integrate the light signals produced by the rods over larger areas of the retina as the illumination level falls. The minimum signal-to-noise ratio required for the detection of a picture detail can thus be maintained during a reduction in scene brightness by several orders of magnitude for correspondingly larger areas on the retina, leading to a gradual decrease in resolving power.

The third effect on the eye of a reduction in scene brightness is that the retina integrates the signal over longer time intervals,

Fig. 1 Typical acuity curve of the eye for a bright stimulus on a black background, together with a straight line expressing the relationship of equation (1).



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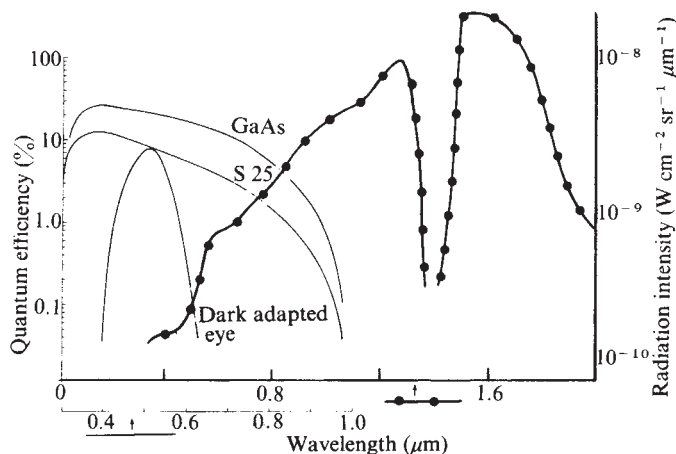


Fig. 2 Typical spectral response characteristic of a dark adapted eye in comparison with a multialkali photocathode of the S25 type, and with a GaAs photocathode^{10,11}. The quantum efficiency curve for the eye is based on the relative energy response curve of ref. 3, p. 73, while assuming a maximum quantum efficiency of 8% at 0.507 μm , as calculated from equation (1) and Blackwell's measurements. Added (---) is a typical spectral distribution of night sky radiation.

up to about 0.2 s, thus again achieving the minimum signal-to-noise ratio required for perception from correspondingly dimmer details, but now at the expense of perceiving movement. A critical flicker frequency can be defined which is linearly related to the logarithm of the stimulus brightness over a wide range of brightness values. This effect is still noticeable at much higher illumination levels, such as that of the fluorescent screen of a television receiver, where an irritating 50-cycle flicker can become apparent if the screen brightness is set too high.

The performance of the eye, like that of any image-forming instrument, can be described with the aid of acuity curves, relating the resolving power (and thus the detection range) to brightness and contrast on a logarithmic scale^{3,4}. If photon noise is the fundamental limitation at the lowest light levels, then these acuity curves can be represented there by relations of the type⁵

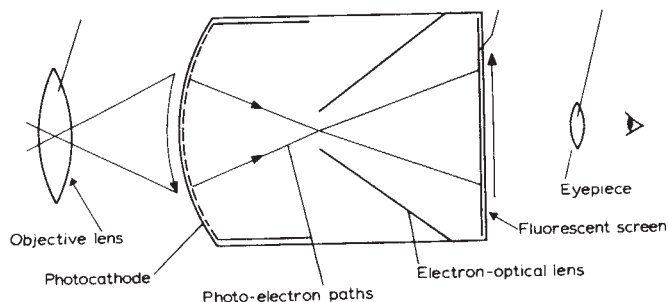
$$L_m \alpha^2 C^2 \tau = \text{constant} \quad (1)$$

where L_m is the mean brightness ($L_m = \frac{1}{2}(L_{\text{high}} + L_{\text{low}})$ in cd m^{-2}) of two adjacent object elements forming the detail with an optical contrast C , defined as $C = (L_{\text{high}} - L_{\text{low}})/(L_{\text{high}} + L_{\text{low}})$, α , the acuity, is the minimum angle (minutes of arc) subtended by each of the two object elements at which their difference in brightness can still be detected, and τ is the integration time.

The constant in the right-hand side of equation (1) can be written as

$$\frac{7.5 \times 10^{10} (S/N)_{\min}^2}{D_{\text{eff}}^2 \theta P}$$

Fig. 3 Schematic diagram of an image intensifier tube.



where D_{eff} is the effective diameter (cm) of the objective lens of the instrument, θ the quantum efficiency of the sensor and P the number of photons per second equivalent to 1 lm of the radiation employed; both θ and P depend on the spectral distribution of the light ($1 \text{ cd m}^{-2} = \pi \text{ lm m}^{-2}$ on a white surface). $(S/N)_{\min}$ is the minimum signal-to-noise ratio required for detection of the object detail. Its value depends on the kind of object detail which the detector must resolve. For some test objects the value of $(S/N)_{\min}$ can sometimes be determined mathematically with the aid of probability theory⁶, by considering the number of alternative choices available and the reliability required in the decision. Blackwell⁷, for instance, has measured acuity curves of the eye by asking observers to look at a circular disk, flashed on to the screen for a short period in any one of eight positions, and requiring a reliability of 50% in the answer. For this situation it can be shown that $(S/N)_{\min} \sim 1.5$.

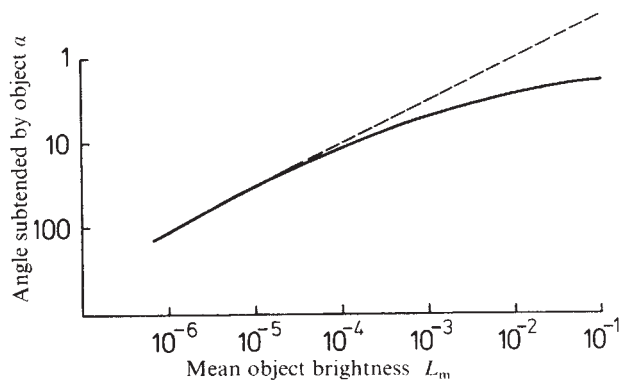


Fig. 4 Schematic acuity curve (arbitrary units) of an image detector. The dashed line is the equation $L_m \alpha^2 C^2 \tau = \text{const}$. At low brightnesses the detector is photon-noise limited; at high brightness the optics limits resolution.

In Fig. 1, a typical acuity curve of the eye is plotted from Blackwell's data⁷ for a high-contrast ($C = 1$) bright stimulus on a dark background. The curve closely approximates the straight line of the photon-noise limited condition, indicated by equation (1), over several orders of magnitude of the light level L_m , with a value of $L_m \alpha^2$ equal to approximately 10^{-2} . If the diameter of the dilated pupil is taken to be 0.76 cm, and a binocular summation factor of 1.26 is assumed⁸ to take into account the use of both eyes in Blackwell's experiments, then D_{eff} can be taken to be 0.85 cm. So for $\tau = 0.2 \text{ s}$ and $(S/N)_{\min} = 1.5$, Blackwell's data indicate a quantum efficiency of the dark-adapted eye of just below 1% for 'white' light with 1 lm equivalent to about 1.3×10^{16} photons s^{-1} .

At 0.507 μm , where the dark-adapted eyes of young people have their maximum, 1 watt is equivalent to 1766 lm (ref. 9). This leads to $P = 1.45 \times 10^{15}$ photons s^{-1} , indicating a maximum quantum efficiency of the dark adapted eye at 0.507 μm of about 8%, according to equation (1). To improve further on the capabilities at low-light levels we may turn to image intensifiers.

Image intensifiers

Instruments of this kind collect more photons from the scene with the aid of a larger lens diameter (up to tens of centimetres) than that of the dark-adapted eye. They use these photons more efficiently, by means of sensors with a larger quantum efficiency than that of the retinal receptors and a spectral sensitivity extending further into the infrared region, as shown in Fig. 2. By so doing they take advantage of the rapid rise in intensity of night-sky radiation (also shown in Fig. 2).

The sensors are image intensifier tubes, first suggested in a patent in 1928 (ref. 12) and illustrated in Fig. 3. The output image can be several thousand times as bright as the image projected on the cathode. An important condition is that the brightness gain is sufficiently high to ensure that an electron created by a photon at the photocathode gives rise to so many photons on the fluorescent output screen of the tube that it will be detected by an observer even if not fully dark adapted.

The performance of the instrument can again be described with the aid of acuity curves, similar to that of Fig. 4, approaching the photon-noise limitation at the lowest light levels and limited by its optical imperfections, as measured by its optical modulation transfer function^{13, 14}, for the smaller picture details. The main improvements over the unaided eye at low light levels are due to the much larger product $D_{\text{eff}}^2 \theta P$ in equation (1).

Two types of image intensifier tube can be considered for modern night viewing equipments, both providing sufficiently high brightness gain to satisfy the condition formulated above. The first, now well established in production instruments, uses so-called cascade tubes¹⁵, illustrated in Fig. 5. Single-stage image intensifier tube modules are stacked in cascade, using fibre-optic coupling between the output screen of one module

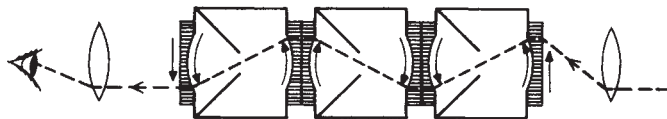


Fig. 5 Principle of operation of a three-stage cascade tube.

and the photocathode of the next. Each fibre-optic window contains millions of glass fibres with diameters of less than 10 μm . Up to three modules are encapsulated together, complete with a miniaturised wrap-around high-voltage supply unit.

High brightness gains can also be achieved by channel electron multiplication¹⁶⁻¹⁹, illustrated in Fig. 6. A hollow tube or channel is shown, usually consisting of a glass with suitable electrical conductivity. When a potential is applied between two end electrodes an accelerating electric field is established down the length of the channel. An electron entering the cathode end of the channel strikes the wall and releases a few secondary electrons. These will be drawn further into the channel under the influence of the accelerating field, and strike the opposite wall at a higher potential, thus releasing further secondaries. The process is repeated several times, giving rise to a large bunch of electrons like an avalanche emerging at the exit for one initial electron.

Since the electron gain depends critically on the length-to-diameter ratio of such a channel and the voltage applied, it is possible to make the individual channels very small and stack them together in a mosaic or channel-plate of a few centimetres diameter and less than a millimetre thick. When such a plate, containing nearly a million channels of 10-15 μm diameter, is placed in front of the fluorescent screen of a single-stage image intensifier tube, brightness gains in excess of 100,000 can easily be obtained. Image tubes of this kind can achieve a better picture quality than cascaded stacks, and lead to smaller and lighter instruments. In addition, channel multiplication has the advantage of local saturation of highlights, owing to the limited supply of secondary electrons available from the wall of each channel. This characteristic substantially reduces the disturbing effect of bright light-sources in the field of view without affecting the gain in darker parts of the scene. The insertion of the channel plate in the paths of the photoelectrons does, however, lead to a loss of some primary electrons which hit the solid web of the plate surrounding each channel, thus missing the entrance to one of the channels.

Channel electron multiplier tubes can be used in small

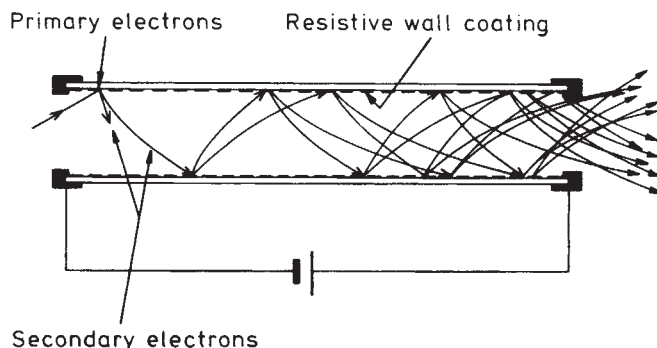


Fig. 6 Illustration of the process of channel electron multiplication.

light-weight goggles, as shown in Fig. 7, and are also suitable for other applications. They can, for example, easily be combined with a television camera tube of the vidicon type to provide a comparatively simple approach to low-light-level television²⁰. In this case the high-light suppression characteristic of the channel tube, and the ease of providing automatic brightness control are important assets. Figure 8 shows photographs taken from the output screen of a channel tube for two different levels of illumination and brightness gain. The picture taken in starlight shows clearly the scintillations corresponding to individual photons.

Thermal imaging

An alternative approach to remote sensing of the environment is to use the natural thermal radiation from the scene. The spectral energy distribution of this black-body radiation is given by Planck's law.

For a black body at room temperature this energy peaks at a wavelength of about 10 μm , with very little energy below 2 μm . Figure 2 showed that the photocathodes used in image intensifier tube have practically no sensitivity at wavelengths above 1 μm . It is therefore not possible to convert thermal images to visible wavelengths with the aid of conventional image intensifier tubes, and it is necessary to replace photoemitters as the primary sensors by detectors of which the spectral sensitivity does extend well beyond 1 μm . These can be photoconductive or photovoltaic cells, where absorbed individual infrared photons can create corresponding charge carriers in the semiconductor material. Alternatively a bolometric effect can be used where the absorbed radiation gives rise to local changes in temperature of the detector material, of which a physical parameter with a high temperature coefficient is measured for every picture element.

A black body of 290 K, that is with a temperature close to

Fig. 7 Photograph of a pair of light-weight goggles, containing channel image intensifier tubes.



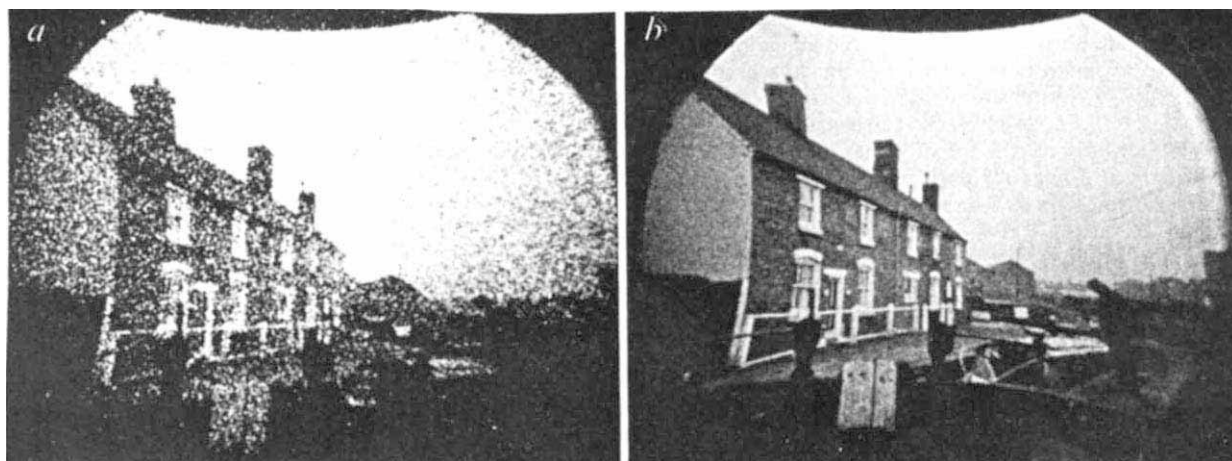


Fig. 8 Photographs taken from the output screen of a channel image intensifier tube, exposure time 1/8 s. *a*, Starlight illumination, brightness gain approximately 100,000. *b*, Full moonlight, brightness gain less than 10,000.

that of most objects in the natural scene, emits approximately 10^{18} photons $\text{s}^{-1} \text{cm}^{-2}$ in the wavelength region up to $20 \mu\text{m}$. (Compare this with the 10^{17} photons $\text{s}^{-1} \text{cm}^{-2}$ in the visible region incident from the sky in sunlight or 5×10^8 photons $\text{s}^{-1} \text{cm}^{-2}$ in moonlight.) The actual numbers of photons available for thermal imaging are thus far larger than those reflected from an object illuminated by the night sky, and even exceed those reflected by sunlit objects by a substantial factor.

In reflected light the image contrast can be very high, exceeding 0.99. But, because the apparent black-body temperature of different natural objects in the nocturnal scene normally differs by not more than about 1°C , the contrast in the thermal image will be very much lower, and depends on the wavelength region used. For example, if all the photons up to $3 \mu\text{m}$ wavelength were used, then the contrast for 1°C temperature difference would be 0.06. This reduces to 0.03 for wavelengths up to $6 \mu\text{m}$, to 0.02 up to $10 \mu\text{m}$ and 0.015 for wavelengths up to $16 \mu\text{m}$.

With such a low contrast, a target composed of a large number of small sensors, arranged in a two-dimensional array, can easily give rise to a serious spatial noise, comparable with a 'dirty window' effect, because of small variations in sensitivity and dark-current of the individual sensors, giving rise to variations in output current comparable to those caused by the thermal signal. The simplest way of avoiding this problem is to use one single sensor cell and scan the image mechanically across it. Spatial variations are thus completely eliminated, but the approach is very uneconomical in its use of the incoming radiation and requires high-speed mechanical scanning.

A compromise solution is to use a linear array of sensor cells

and scan the thermal image of the scene mechanically across this array in one or more sweeps by wobbling the optics, to constitute the frame scan. The output of each cell in turn can be sampled at high frequency in order to create the conventional video signal. By limiting the number of sensors to at most a few hundred, it is still possible to select arrays with only small differences in dark current and sensitivity of individual sensors, or even to correct for these.

The performance of such thermal imaging systems, using arrays of n sensors, is again noise limited according to a relationship which can readily be derived from the one calculated by Chiari and Jervis²¹

$$\Delta T_N \alpha = \text{constant} \quad (2)$$

where ΔT_N is the noise equivalent temperature difference, that is, the difference in black-body temperature between two object elements in the scene for which the corresponding signal component of the detector output current just equals the combined noise current of all the cells and α is the angular resolution (in radians). The constant describes system characteristics and can be written as $4(f/D^2)(p/n)^4(1/M^*)$ where D is once again the diameter of the objective lens (in cm); f its focal length; p the number of cells scanned per second (equivalent to the bandwidth of the video signal) and M^* the figure of merit of the detector material, indicating the sensitivity of the detector to differences of temperature in the scene. The figure of merit also takes into account a given atmospheric absorption of the infrared radiation.

Table 1 gives typical values of M^* for a number of detector

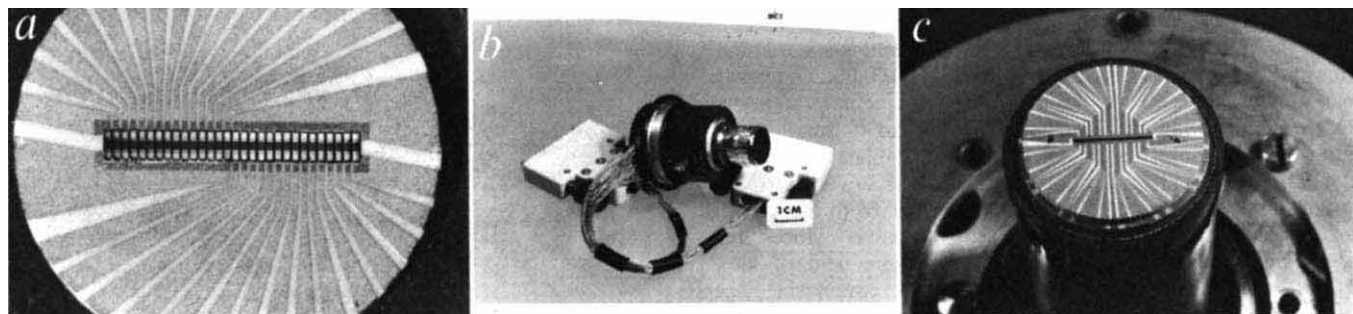


Fig. 9 Photographs of linear arrays of CMT cells, courtesy F. D. Morten of Mullard Limited, Southampton. *a*, 30-element array of CMT cells, $115 \times 125 \mu\text{m}$. *b*, As (*a*) mounted. (*c*) 192-element array of CMT cells, $50 \times 50 \mu\text{m}$.

systems produced in arrays, allowing for typical transmission at a range of 2 km (ref. 22). For the smaller values of α the just perceptible temperature differences in the scene, given by equation (2), will again be restricted by the degradation in resolution caused by the optical components. Equation (2) does, however, indicate that high-performance thermal imaging is feasible with linear arrays of sensor cells. Recognisable images of a natural scene can be formed when temperature differences do not exceed about 1 °C. It should, for example, be possible to obtain television-type pictures with a temperature

with an array of approximately 150 photoconductive cadmium mercury telluride (CMT) cells ($M^* \sim 3 \times 10^6$) at 77 K, provided that the optics are not yet limiting the resolution.

Sensors of this kind can now be manufactured in arrays of up to several hundred individual elements with sizes typically down to about 50 μm . Arrays of 30 and 192 CMT sensor elements, with leads connecting bonding pads at the edge of the carrier directly to the elements, are illustrated in Figure 9.

The photographs of Figure 10, courtesy of Mr. W. D. Lawson of RSRE, Great Malvern, illustrate the type of thermal



Fig. 10 Thermal pictures obtained with 30-element CMT arrays. *a*, A housing estate in daytime; *b*, A small fishing boat at night.

resolution of 0.1 °C, together with an angular resolution of 0.2 mrad and a total viewing angle of about 7°, for an objective lens with $f = 30$ cm and $D = 20$ cm, a picture of 400×600 picture elements and 25 frames per second, if a value for $M^*n^{\frac{1}{2}}$ of about 3.67×10^7 is achieved. This should be obtainable

picture obtainable in the 8–13- μm band with 30-element arrays of CMT, cooled to 77 K. The angular resolution of the equipment used was 0.25 mrad, the field of view $10^\circ \times 4^\circ$ and the frame rate 25 pictures per second. Arrays and individual cells for thermal imaging systems with lower temperature and angular resolution are also commercially available. These include CMT cells for operation at intermediate temperatures of 193 K to 233 K. These temperatures can be achieved with the aid of comparatively small and compact thermo-electric coolers²³.

A somewhat modified approach to thermal imaging with linear sensor arrays is particularly suitable for airborne photography. In this case an image of the terrain is swept across the sensor or sensors to trace the lines of the picture, whereas the movement of the aircraft provides the much slower continuous scanning motion in the other direction. By recording the video information on a film with continuous uniform movement, a picture of a strip of terrain is gradually built up with high temperature and spatial resolution²⁴.

An interesting alternative to cooled arrays of photocells is to translate the thermal image into a pattern of electrical charges

Table 1 Typical values of M^* for detector systems produced in arrays

Detector systems available	Element temperature	Frequency (Hz)	M^* (cm^{-1} , $\text{Hz}^{\frac{1}{2}}$, K^{-1})
CMT (30 elements)	77 K	5000	4×10^6
CMT (192 elements)	77 K	5000	3×10^6
TGS (128 elements)	295 K	800	10^4
TGS (128 elements)	295 K	125	2×10^4
TGS single element	295 K	800	2×10^4
CMT 3–5 μm band	233 K	5000	10^5
CMT 8–13 μm band	233 K	5000	3×10^4

CMT, cadmium mercury telluride. TGS, triglycine sulphate. M^* is for a 2-km atmospheric transmission path.

on a two-dimensional target, and to read these out with the aid of a scanning electron beam, as in a television pick-up tube of the vidicon type, illustrated in Figure 11. Wherever the photoconductive material, which can for example be PbTe (ref. 25) receives infrared radiation, charge can leak to the surface from the positive semi-transparent electrode underneath. The electron beam removes this charge during the next scan, thus creating a signal-current proportional to the local level of illumination.

Instead of using a photoconductive material, it is also possible to make use of the pyroelectric effect²⁶ exhibited by some materials, such as for instance triglycine sulphate (TGS) or one of its derivatives. Thin slices of such a material acquire electrical charges on the surfaces, whenever the temperature of the material changes. This effect has led to the

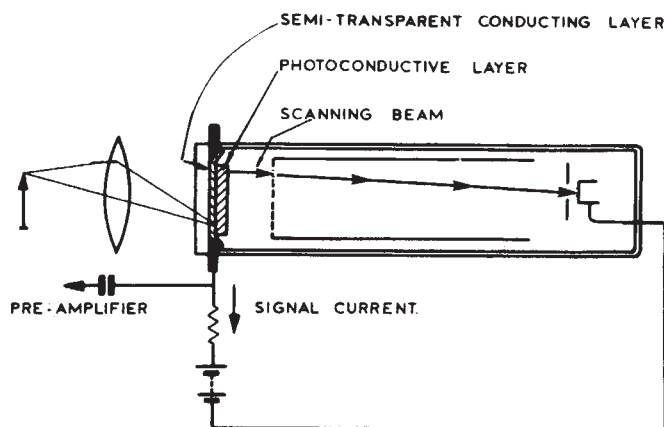


Fig. 11 Schematic diagram of a vidicon.

development of the pyroelectric vidicon^{27,28}. Such a tube can translate thermal images of the natural scene into useful video signals with temperature resolutions already down to about 0.2 °C for a 100-line picture.

Further improvements of this approach are still envisaged, both in terms of target design^{29,30}, and more suitable pyroelectric materials^{31,32}, which might eventually make it competitive with some of the smaller linear arrays of cooled CMT sensors, but with the advantages of more simplicity, operation at room temperature and not requiring high-speed mechanical scanning.

Whereas image intensification as a means for improved night viewing is now quite close to its theoretical photon-noise limited optimum performance, considerable further improvements in thermal imaging techniques can still be foreseen. These can be expected to result partly from further advances in detector fabrication technology, such as monolithic sensor arrays prepared on chips of single crystal material³³, or even two-dimensional arrays with integral charge-coupled read-out³⁴⁻³⁸.

Second, recent advances in signal processing, first developed for improving pictures obtained in the American space programme, can also make a major contribution to increasing the picture quality in thermal imaging, by means of processes like edge enhancement, deblurring, contrast manipulation and extracting small picture signals from noise³⁹.

Remote sensing methods of this kind, using thermal imaging, are likely to provide a night vision capability to military and security forces⁴⁰⁻⁴³ at longer ranges than can be realised with image intensifiers, partly also because objects of special interest in the scene, like vehicles with their engines running and

human bodies, are usually a few tens of degrees hotter than their natural surroundings.

Other applications of thermal imaging

Thermal imaging has so far received its main stimulus as an alternative night viewing aid to image intensifiers for military applications. The thermal pictures received from the scene do, however, provide different information to that normally obtained with reflected visible light, and are therefore also beginning to prove their usefulness in a wide variety of other applications⁴⁴⁻⁴⁷. These include geological surveys to map water resources, indicate potential oil and mineral deposits, as well as agricultural inspections to monitor crop development and detect early signs of crop failure due to fungus growth or other diseases.

Other applications are in the fields of medicine⁴⁸⁻⁵⁰ (thermography), where it may also sometimes be useful to add isothermal contours to the picture^{51,52}, meteorology, urban planning, energy conservation by optimising the thermal insulation of dwellings, monitoring environmental chemical and thermal pollution, oceanography, astronomy, etc. In some cases the thermal band is divided into a number of separate channels to provide multispectral data^{53,54}, which can be suitably processed and used to reproduce multicolour pictures in the visible spectrum, thus facilitating even further the identification of particular terrain features with uniquely different spectral characteristics.

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articles

The observability of possible atmospheric removal processes for chlorfluorocarbons 11 and 12

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The existence of tropospheric sinks for CFCs 11 and 12 would have a large impact on projected stratospheric ozone effects. The existence of such sinks, distinct from no sinks, cannot at present be discerned from atmospheric measurements. As presently analysed, tens of years may pass before such a distinction would be possible. This article scrutinises transient mechanisms that could contribute to accomplishing this critical distinction in 10 yr or less.

THE issue of possible stratospheric ozone destruction by halo-carbons, and specifically chlorfluorocarbons 11 and 12 (CFCs 11 and 12), depends sensitively upon the presence or absence of tropospheric sinks, as indicated by several theoretical studies¹⁻⁷ using steady-state and transient one-dimensional (1-D) parameterised transport calculations. These studies focus on: (1) atmospheric CFC mixing ratios for assumed continuing CFC production; (2) Stratospheric ozone depletion for assumed continuing and halted CFC production.

Notably absent, and the subject of this article, is a direct examination of the information content in transient atmospheric CFC ratios following an assumed abrupt reduction of one or more CFCs.

In analysing relatively weak CFC loss mechanisms, weighed against their production and atmospheric transport processes, the present status of the problem is as follows: photodissociation losses of CFCs 11 and 12 above 30 km are generally established⁸; consensus has not been reached on the existence, or non-existence, of other significant loss mechanisms for CFCs 11 and 12⁹⁻¹⁰; observational data^{10,11} are sparse, variable and uncertain^{9,11}; 1-D theoretical results are uncertain and globally unresolved^{8-10,13,14}; CFC production and release data have been extensively compiled and updated^{9,12}; and observational CFC mixing ratio data, sparse at present, are growing with increasing efforts towards absolute calibration and accounting of local background effects⁹⁻¹¹.

Estimates have been made of the potential reductions⁸⁻¹⁰ of asymptotic CFC mixing ratios that could occur from other atmospheric loss processes acting in addition to CFC photodissociation. Many decades must pass, however, before asymptotic conditions will occur—even if significant tropospheric removal processes do exist.

This article suggests previously unnoted transient mechanisms and trend analysis that, with appropriate development, could contribute to a resolution of this critical issue of tropospheric losses in 10 yr or less.

Calculation procedure

The purpose of our analysis is to assess the physical observability of tentative tropospheric CFC loss processes, without a

detailed consideration of complex chemical mechanisms. Our investigative examples employ a conventional 1-D time-dependent representation of the Earth's atmosphere from 0 to 55 km with arbitrary CFC production and loss processes. Atmospheric transport is represented by the computational artefact of vertical eddy diffusion. The descriptive equations for globally averaged concentrations, $C_i(z,t)$ cm⁻³, are given by

$$\frac{\partial C_i}{\partial t} = \frac{\partial}{\partial z} \{ K(z) \rho \left[\frac{\partial (C_i/\rho)}{\partial z} \right] \} + P_i - L_i \quad (1)$$

$i = \text{CFCs 11 and 12}$

The vertical eddy diffusion coefficients are denoted by $K(z)$ and, for illustrative purposes, are assigned the values used by

Fig. 1 Relative tropospheric volume mixing ratios for CF₂Cl₂ (CFC 12). —●—, no sink; —○—, 10-yr tropospheric sink. *a*, Source constant after 1974; *b*, source = 0 after 1974.

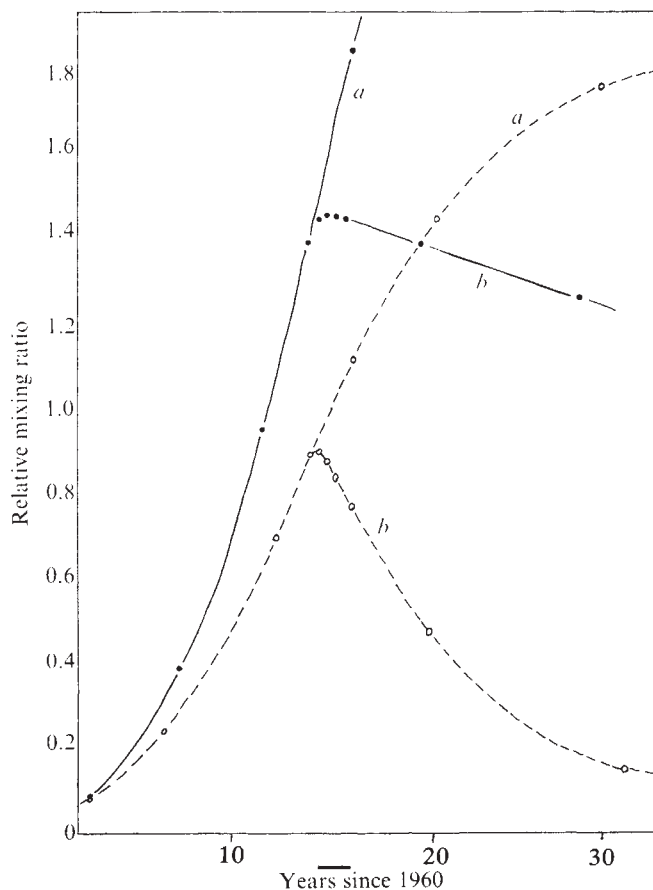


Table 1 CFC mixing ratio trends for the 15–20-yr interval

CFC	Lifetime of assumed tropospheric sink (yr)	Trends for source constant after 1974	Trend ratio (10 yr)/(∞)	Trends for source = 0 after 1974	Trend ratio (10 yr)/(∞)
CFC1 ₃	∞	0.1	~½	-0.02	~2
	10	0.06		-0.04	
	∞	0.2		-0.01	
CF ₂ Cl ₂	10	0.08	~½	-0.07	6–7
	∞				

Chang¹³. Comparative results with other sets of $K(z)$ appear in refs 9, 15. Natural and anthropogenic CFC production⁹ and loss terms are denoted by P_i and L_i , respectively. The atmospheric number density, ρ , is taken from the US Standard Atmosphere¹⁶, and atmospheric mixing ratios are

$$\mu_i(z,t) = C_i(z,t)/\rho(z,t), \quad i = \text{CFCs 11 and 12} \quad (2)$$

Loss rates are expressed as

$$L_i = J_i C_i + (1/\tau_i) C_i, \quad i = \text{CFCs 11 and 12} \quad (3)$$

The photodissociation rates, J_i , are taken from Rowland and Molina⁵. The following additional CFC loss processes will be examined: (1) 10-yr tropospheric sink ($\tau_i = 10$ yr for 0–15 km); (2) 40-yr tropospheric sink ($\tau_i = 40$ yr for 0–15 km); (3) 10-yr stratospheric aerosol sink ($\tau_i = 10$ yr for 15–20 km).

Our calculational procedure was first applied to parameterised transport models with arbitrary sources and sinks by MacCracken and Gelinas^{17–19}, and extended to include complex photochemical mechanisms by Chang *et al.*²⁰ This method of casting reactive transport equations into ordinary rate equations for fully implicit, simultaneous integration in space and time is extensively documented, with supportive motivations and assumptions^{17–23}. Vertical mesh spacings of 1 km were used, and time-dependent release rates⁹ were converted to corresponding globally averaged, time-varying source rates in

the 0–1 km zone. Upper boundary conditions of both zero net flux and zero re-entrant flux at 55 km yield the stated results.

Results

Cases (1)–(3) focus directly on circumstances where tropospheric CFC removal processes would be most immediately observable, that is, with inconsequential CFC production, relative to losses. Either abrupt source reductions or total curtailment of CFC 11 and/or 12 can be studied. Total source curtailments are used in our examples because the information content of governing mechanisms is most readily apparent in that limit.

Our primary results are developed from Fig. 1, which presents the relative mixing ratios of infinite against 10-yr, tropospheric lifetimes for CFC 12 with: source rates continuing constant at 1974 levels, subsequent to 1974; or production assumed halted subsequent to 1974. Also calculated, but not plotted, are CFC 11 results which have the same qualitative structure as CFC 12 results. Quantitative differences are noted in Table 1. Also, results for a 40-yr tropospheric sink and a 10-yr stratospheric aerosol sink were calculated, and lie between the 10-yr and infinite lifetime results.

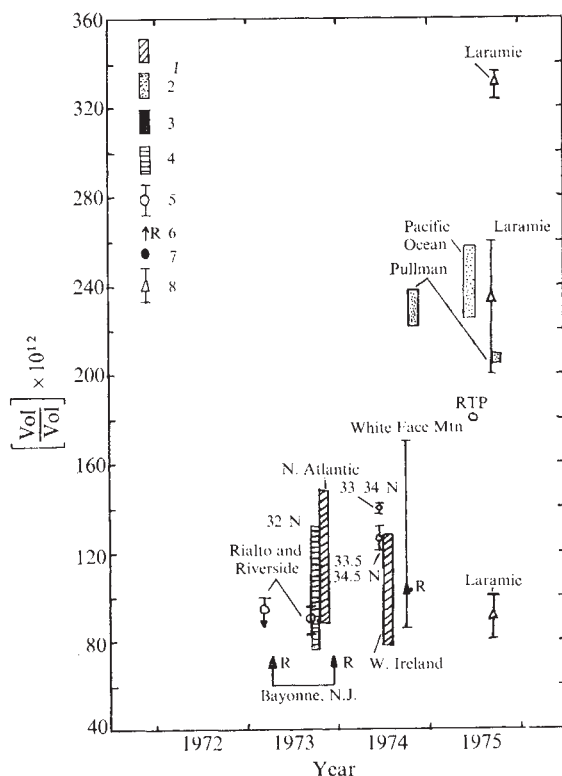
Our analysis now concentrates on relative trends in an effort to minimise the role of absolute uncertainties and poor global resolution in observations and calculations. The following points can be noted: (1) Following curtailments linear trends are immediately established and persist for nearly a decade. (2) The tropospheric trend ratio (Table 1) for curtailed CFC 12 is distinctly different (6–7 against ~2) from that for the other cases, which may present observational and interpretive opportunities for distinguishing any significant tropospheric sinks. (3) Tropospheric trend significance of $\pm 10\%$ may be necessary to distinguish significant tropospheric sinks.

Figures 2 and 3 present existing observational data that seem most representative of background CFC levels^{9,11}. We present these data primarily to view trend significance, which is no better than $\pm(25\text{--}50\%)$, even accounting for northern-southern hemispheric decrements¹⁰. In fact, because this existing trend data, as well as absolute CFC mixing ratio magnitudes, have sufficient scatter, it is possible that CFC production and release is greater than currently reported and estimated^{9,10}.

Conclusions

Our investigation was prompted by the prospect of continued indeterminacy of critically significant tropospheric CFC sinks from absolute analyses, and the practical need to make the best use of somewhat randomly collected, sparse and often uncalibrated observational data, coupled with available 1-D theoretical interpretations that incorporate uncertain transport parameterisations with no horizontal resolution. We found, following a source curtailment, that the trend of tropospheric CFC 12 mixing ratios is different by nearly an order of magnitude for 10-yr tropospheric sinks, against no tropospheric sinks. Furthermore, these calculated trends are distinct almost immediately following a source curtailment. Considerations for reducing this mechanism to practical application suggest a number of related points: (1) Concentration on tropospheric measurements may suffice for determining critical CFC sinks, irrespective of atmospheric chemistry details. (2) Tropospheric measurements at permanent, stationary stations would be useful for trend analyses, preferably at several northern and southern

Fig. 2 Observed tropospheric mixing ratios for CF₂Cl₂ (CFC 12). Sources: 1, Lovelock; 2, Washington State University; 3, Heidt *et al.*; 4, Naval Research Lab.; 5, University of California, Riverside; 6, Rutgers University; 7, EPA; 8, Golan *et al.*



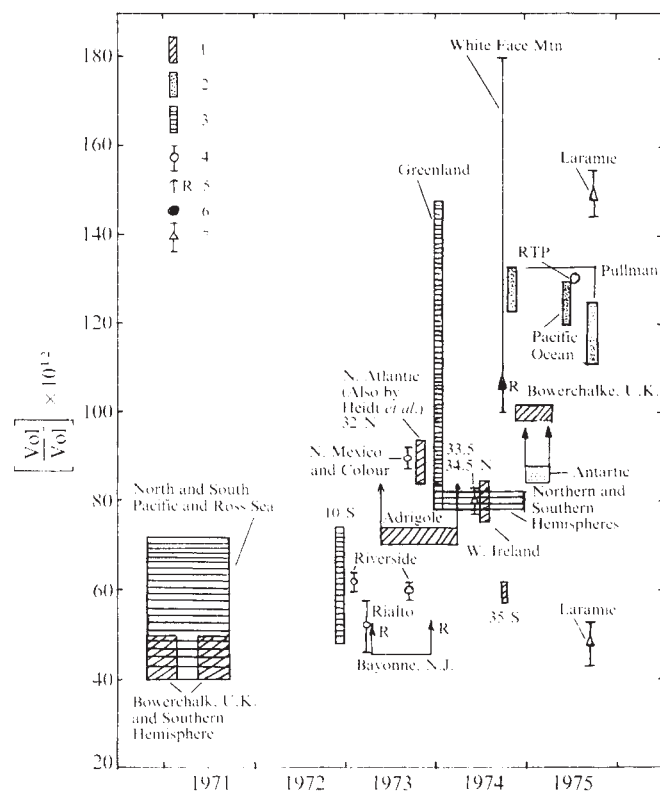


Fig. 3 Observed tropospheric mixing ratios for CFC_{12} (CFC 11). Sources 1-7, as in Fig. 2 legend.

latitudes, with many observations taken daily. Siting should maximise the probability of measuring CFC background levels. (3) Observations at each station should include selected tropospheric altitudes, and an array (north, south, east and west) of horizontal sites which may provide a needed measure of local effects. (4) Meaningful trends, inclusive of seasonal variations, could be evaluated station by station, and further processed for both latitudinal and global significance. (5) Absolute calibration for CFC measurements should be established. (6) Natural variabilities in trend data could prove to be too large for 1-D analysis to apply with adequate sensitivity for an explicit determination of possible tropospheric FC sinks. If so, this realisation would be of greatest value earlier, rather than later.

Since this avenue of dynamic analysis is only now put forth for CFCs, a substantial amount of possible future investigation comes immediately to mind. More incisive considerations of CFC data significance and interpretability may be accomplished by analysis of local, meridional and hemispheric trends, in association with global evaluations. These considerations may extend to further analysis of dynamic responses to effective eddy diffusivity variations in 1-D and 2-D, to general atmospheric circulation mechanisms, and to natural radiative variations^{24,25}.

Several circumstances of gradual against abrupt changes in CFC production, release or substitutions remain for extended investigation. Correlated and anticorrelated trends of possible CFC substitutions could offer very effective filtering of absolute uncertainties, consistent with the mechanisms presented above.

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Direct demonstration of β -globin mRNA in homozygous Ferrara β_0 -thalassaemia patients

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In cases of β_0 -thalassaemia from Ferrara, Italy, the β -globin gene is transcribed into mRNA but no protein is synthesised. For these cases there is no hybridisation data suggesting a globin gene structural mutation. This again demonstrates the diverse molecular events which may cause this prevalent hereditary disease.

β -THALASSAEMIA is an hereditary anaemia in which there is an excess of α -globin over β -globin chain synthesis. In homozygotes for β_0 -thalassaemia no β -globin is synthesised, and active haemoglobin is only obtained by the compensatory synthesis of δ - and γ -globin chains. The wide geographical distribution and varying clinical severity of β -thalassaemia indicates the existence of several different lesions at the molecular level,

each of which could give rise to the reduced synthesis of β -globin chains¹.

Several cases of β_0 -thalassaemia have been described for which there is an absence or reduction in β -globin messenger RNA (mRNA _{β}) (refs 2–6), as judged by protein synthesis in a cell-free system or by DNA-RNA hybridisation. Cases from Ferrara, Italy, seem to differ from this more usual pattern, in that it has been reported that β -globin chain synthesis can be activated in reticulocytes both *in vivo* after transfusion and *in vitro* by the addition of post-ribosomal supernatant from normal reticulocyte lysates^{7,8}. But so far it has not been demonstrated conclusively by direct measurements that mRNA _{β} is present in such a patient.

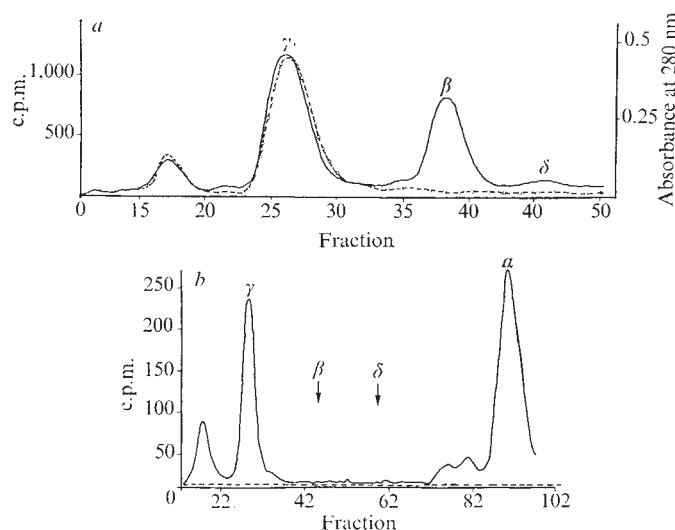


Fig. 1 Globin synthesis in whole cell incubations of reticulocytes from two Ferrara patients. In both cases reticulocytes were incubated with ³H-leucine for 1 h at 37 °C; chain isolation and analysis were carried out on carboxymethylcellulose columns as described previously⁸. Carrier adult globin was added in one case (a, patient FR); in the other case (b, patient AS) the position of the β - and δ -globin peaks were determined in a parallel run. In both patients, as for the other cases examined, less than 1% of the total amino acid incorporation into globin chromatographs with the β -globin peak. These experiments were performed, in some cases, simultaneously in two laboratories with identical results.

Techniques for preparing complementary DNA (cDNA) probes which can be purified for homology for β -globin genes and mRNA⁴ have enabled us to assay directly by hybridisation for mRNA _{β} in several patients with Ferrara thalassaemia both before and after transfusion. Significant levels of mRNA _{β} (up to 12%) have been detected in circulating reticulocytes of patients for whom globin chromatography demonstrates the absence of β -globin chain synthesis. The level of β -globin-specific mRNA sequences in nuclear RNA from the one patient studied is higher still, between 20 and 30%. Ferrara thalassaemia thus differs in molecular pathology from many southern Italian and Asian β_0 -thalassaemias, for which the β -globin gene is present but no detectable mRNA is found in the reticulocyte⁵, and from South-east Asian α_0 -thalassaemia, which is caused by an α -globin gene deletion^{9,10}. In other cases of β_0 -thalassaemia mRNA _{β} is present, but cannot be translated^{23,27}.

The conclusions drawn from the DNA-RNA hybridisation experiments will depend on the total absence of β -globin chain synthesis in the cases studied. In each case the total haemoglobin prepared after red-cell lysis was chromatographed on Amberlite and haemoglobin F was eluted¹¹. The remaining haemoglobins A and A₂ were eluted separately and estimated by absorbance.

The sensitivity of this technique allows estimation of as little as 0.2% haemoglobin A in the presence of haemoglobins F and A₂. In several cases studied the circulating red blood cells were incubated with ³H-leucine as previously described⁷ and the globin chains were extracted and separated on carboxymethylcellulose¹². This technique is less sensitive but makes possible a direct estimate of β -globin synthesis; as little as approximately 1% β -globin chain can be detected.

In both cases described here there was no haemoglobin A detected on Amberlite chromatography (AS, FR, FB, SM, AM, GC) or on paper electrophoresis (PS) before transfusion. Globin chain chromatography also demonstrated the absence of β -globin chain synthesis (Fig. 1). Therefore it can be concluded, in agreement with our previous findings, that by normal rigorous criteria these patients have a β_0 -thalassaemia¹³; γ - and δ -globin synthesis occurs but no β -globin chains are made.

cDNA was prepared as previously described using human globin mRNA as template, oligo(dT) as primer, avian myeloblastosis virus RNA-dependent DNA polymerase as

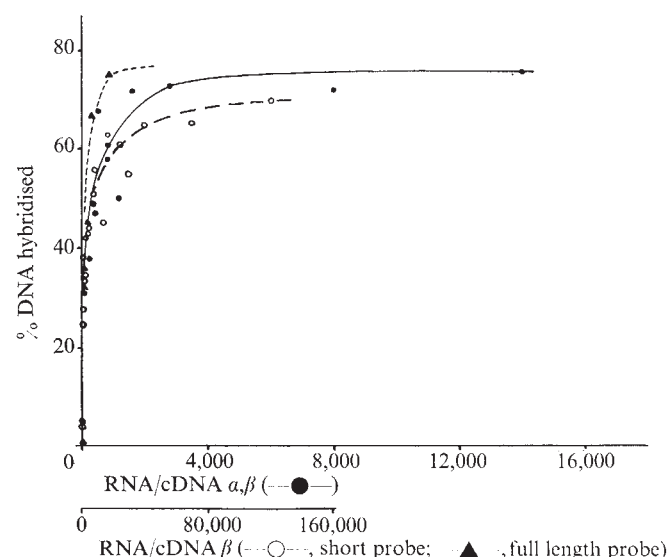


Fig. 2 Titration of cDNA _{β} and cDNA _{α,β} with Ferrara thalassaemia reticulocyte RNA. RNA was prepared from ribosomal materials⁸ or by direct phenol-chloroform extraction of total circulating red cells as described before^{4,5}. Each RNA sample was titrated with cDNA _{α,β} (cDNA _{α} , approximately 55–60%; cDNA _{β} , approximately 40–45%) prepared from normal adult reticulocyte globin mRNA, and with cDNA _{β} (approximately 80–85% pure) prepared from HbH disease (α -thalassaemia) reticulocyte globin mRNA. The specific activity of 'short' and full size cDNA_{HbH} (cDNA _{β}) and the cDNA _{α,β} used as probes was 14,000 c.p.m. ng⁻¹ at a counting efficiency of 30%. In the hybridisation conditions used plateau levels are reached between 70 and 80% with normal globin 9S RNA. A constant amount of cDNA _{α,β} or cDNA _{β} (18–35 pg) was hybridised with increasing amounts of Ferrara β_0 reticulocyte cytoplasmic RNA, in a volume of 1–2 μ l of 50% formamide, 0.5 M NaCl, 25 mM HEPES buffer, pH 6.8, 1 mM EDTA, containing *E. coli* RNA (2.5 mg ml⁻¹). Samples were sealed in silicone-coated glass capillaries, and incubated at 43 °C for 12–14 d. The percentage of cDNA hybridised was determined by assay with single-strand-specific S1 nuclease, followed by perchloric acid precipitation of hybridised material. Further details are given in previous publications^{4,5,9,15}. The ratio mRNA _{β} :mRNA _{α} was calculated by adjusting the abscissa until the initial portions of the hybridisation curves were superimposable, and (after adjusting for the amount of cDNA _{β} present in the cDNA _{α,β}) taking the ratio from the magnitude of this adjustment. With normal adult globin mRNA the mRNA _{β} :mRNA _{α} ratio obtained by this technique is approximately 0.65. In this case (MA) the ratio mRNA _{β} :mRNA _{α} is 0.05–0.07. Calculations made from initial slopes of hybridisation gave similar values. When the amount of β -mRNA in a preparation (relative to α -mRNA) is as low as 1–20% the initial slope of the hybridisation of the cDNA _{α,β} essentially reflects the hybridisation of the cDNA _{α} component.

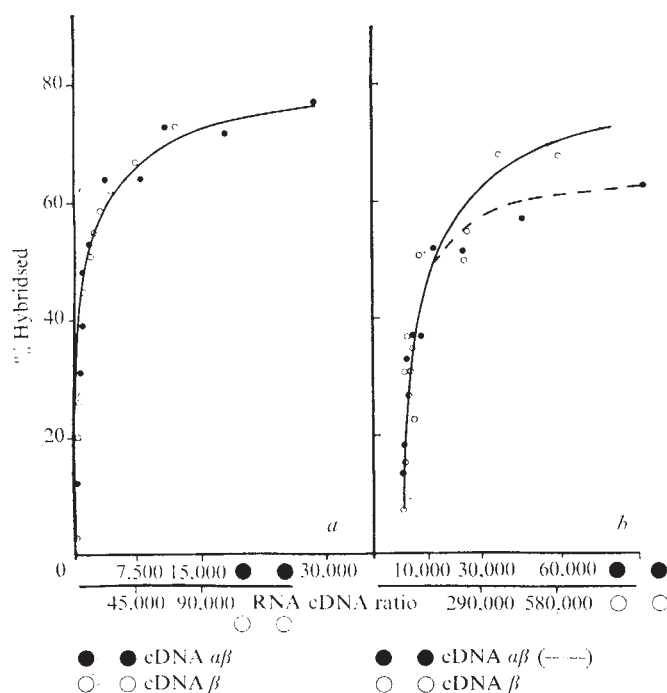


Fig. 3 Titration of bone marrow total nuclear and cytoplasmic RNAs. Cytoplasmic (a) and nuclear (b) total RNA was prepared from iliac crest bone marrow using a citric acid procedure as described before¹⁵ and titrated with cDNA_{αβ} and cDNA_β (short probe) as described for Fig. 2. In this case (SM) the ratio cDNA_β:cDNA_α is 0.10–0.15 for cytoplasmic RNA and 0.20–0.30 for nuclear RNA. The ratios required for saturation are much higher for bone marrow than for reticulocytes because of the considerably lower proportion of globin mRNA present¹⁵.

is already increased to approximately 80–85% (mRNA_β). These mRNAs were transcribed to give cDNA_{αβ} and cDNA_{HbH}, which were used directly as probes for mRNA_α and mRNA_β sequences in various RNA preparations from the patients with β₀-thalassaemia (Ferrara). The proportion of mRNA_β relative to mRNA_α was estimated by comparing the initial slopes of the hybridisations with the cDNAs by adjusting the scale of the x axes to make the two hybridisation curves superimposable. The untransfused patients studied (three cases) showed mRNA_β levels from 2 to 12% in reticulocytes as a proportion of mRNA_α (Fig. 2). In four patients mRNA_β was determined 3–4 weeks after transfusion, when β-globin synthesis was demonstrable in intact reticulocyte incubation. In three of these cases the reticulocyte mRNA_β levels were between 10 and 20%. In the fourth case a bone marrow sample was examined; the proportion of nuclear RNA sequences hybridising to cDNA_β was found to be two or three times higher than in cytoplasmic RNA (Fig. 3). The level of mRNA_β in the circulating reticulocytes of the same patient 5 weeks after transfusion, when β-globin synthesis in intact erythrocytes had become undetectable, was 2%. The results are summarised in Table 1.

The methods for estimating the absolute amounts of mRNA_β relative to mRNA_α are somewhat imprecise, particularly because the calculation depends on the difference between hybridisation to cDNA_β and to cDNA_{αβ} and might give a maximum error of as much as 30% for each value determined. But the fact that the hybridisations go to completion compared with hybridisations to normal reticulocyte RNA for each probe at usual low RNA:cDNA ratios demonstrates the presence of sequences indistinguishable from mRNA_β by hybridisation criteria in circulating reticulocytes from these cases of β₀-thalassaemia. In this respect we wish to stress that, even when a full size cDNA_{HbH} is used, protection of this probe from S₁ nuclease to the same extent as with normal mRNA_{αβ} is obtained. Even allowing for errors, the mRNA_β

Table 1 mRNA_β content for different patients

Patient		%HbA*	%mRNA _β † (in reticulocytes)		
Never transfused	AS	<0.2	10–12		
	FR	<0.2	2–3		
	AM	<0.2	2–3 (5–7‡)		
Transfused		% β-Globin synthesis	% mRNA _β † (in reticulocytes)		
	PS	31.3	10–20		
	FB§	22.5	12–50		
		14.4	15–20		
	GC	30.8	11–15		
	SM	18.5	—		
				% mRNA _β † in bone marrow cells	
				cytoplasm	nuclei
				10–15	20–30

β-Globin synthesis was determined 3–4 weeks after transfusion in intact reticulocyte incubations⁶. The values reported are relative to α-globin synthesis.

* Relative to total haemoglobin.

† Relative to mRNA_α. In all experiments reported, cDNA_{HbH} hybridisation went essentially to completion (68–80%).

‡ Determined on RNA extracted from ribosomal material⁸.

§ Determinations were on blood samples taken after two different transfusions.

enzyme, and ³H-dCTP as the only labelled deoxynucleotide triphosphate^{1,5,9,15,16}. For most of the experiments reported, 'short' cDNAs (complementary to the 3'-end half of the mRNA) were prepared; however, in a few instances full size cDNAs were used¹⁷. Human globin mRNA was obtained from reticulocytes from a subject with a non-thalassaemic haemolytic anaemia (mRNA_{αβ}) and from a patient with haemoglobin H disease (α thalassaemia), in which the proportion of mRNA_β

levels determined are very much higher than the maximum level of β-globin chain synthesis which might have remained undetected, if any, in the untransfused cases (Fig. 2). No mRNA_β could be detected in identical experiments run in parallel with cytoplasmic RNA from two non-Ferrara cases of β₀-thalassaemia, one of which was examined 3–4 weeks after transfusion⁵; levels of less than 1% of β-mRNA-like sequences have been found consistently in a series of 10 β₀-thalassaemic patients of

non-Ferrara origin (P.C., B.G. and S.O., unpublished observations); thus a contribution of mRNA_δ to hybridisation could not explain the results obtained, as the haemoglobin A₂ levels were similar in all cases.

The thermal stability of the hybrid between Ferrara RNA (from transfused and untransfused patients) and cDNA_{HbH} was 2 °C lower than that of the hybrid between normal 9S mRNA and cDNA_{HbH} (Fig. 4). This confirms that the hybridisation observed is due to mRNA_β sequences or sequences very similar to mRNA_β; the smooth form of the melting curve argues for accurate hybrid formation along the entire sequence. The nucleotide divergence corresponding to a ΔT_m of 2 °C is between 0.5 and 1.0%¹⁸⁻²¹; however, the authors are not sure the difference is significant, as thalassaemic mRNA samples are often degraded owing to difficulties in collection. An effect on melting temperature of this magnitude has been demonstrated

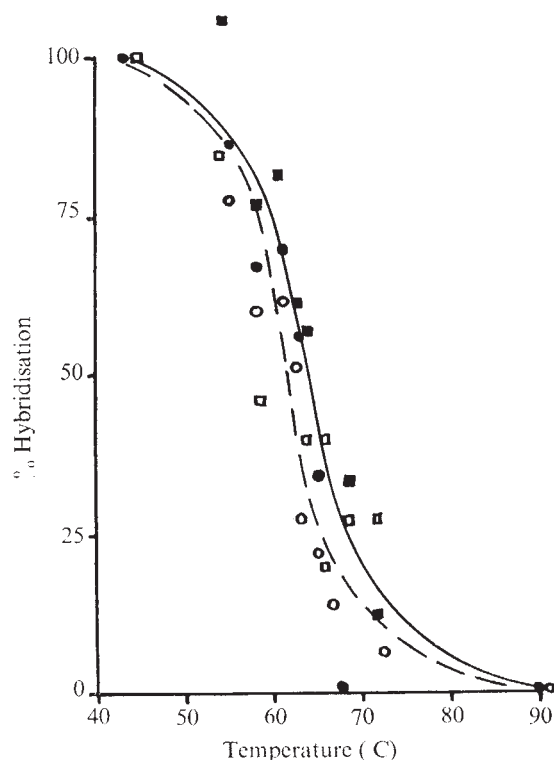


Fig. 4 Melting profiles of normal and Ferrara globin mRNA to cDNA_β. 20 pg 'short' cDNA_{HbH} were hybridised in individual capillaries with 400 pg normal globin 9S RNA or either 0.9 μg total RNA (AM, ribosomal) or 5.6 μg total RNA (GC) in 1 μl hybridisation buffer at 43 °C for 13 d. Both AM and GC were shown to be β₀ thalassaemics by Amberlite chromatography. At the end of the incubation, capillaries were heated to the indicated temperature, quickly cooled to 0 °C, diluted with S1 nuclease assay buffer and frozen until assayed with S1 nuclease. Data are expressed as percentage of maximum hybridisation obtained; background S1 resistance is subtracted. In this experiment cDNA_{HbH} hybridised to 73% with normal 9S mRNA and to 65% with Ferrara thalassaemia RNA. ● and ■, Normal RNA; ○, RNA from GC; □, RNA from AM.

for the hybridisation of short rabbit globin mRNA transcripts to cDNA_β²². The melting behaviour of a cDNA_β-mRNA_δ hybrid is not yet known.

Hybridisation to chain-specific cDNA demonstrates the presence of homologous mRNA sequences, and because there is little evidence of cross hybridisation in the globin gene system^{4,5}, these sequences demonstrated with cDNA_β arise from transcription of the β-globin gene. In the cases considered

at least some complete β-globin mRNA molecules must be present, for full size cDNA_{HbH} can hybridise to completion to Ferrara mRNA.

Attempts to demonstrate synthesis of β-globin directed by isolated mRNA in a heterologous cell-free system have been disappointing, although Pritchard *et al.*¹⁴, using RNA from patient BS, reported the synthesis of a fraction with the chromatographic characteristics of β-globin chains. Because of insufficient radioactivity in the peptides Pritchard *et al.*¹⁴ were not able to identify the peak which chromatographs with β globin unequivocally by fingerprint analysis. Further experiments to this end will be undertaken when enough Ferrara thalassaemia mRNA becomes available.

In Ferrara thalassaemia, apparently normal mRNA_β found in erythroid cells which hybridises to cDNA_β, can be activated *in vitro* by the addition of normal post-ribosomal supernatant⁷ and can be activated *in vivo* by transfusion with normal blood⁶; the latter finding has been confirmed in the patients studied in the work reprinted here. The untranslated β-globin mRNA apparently decays faster than normal, as indicated by the higher level of mRNA_β found in bone marrow relative to reticulocytes, and by the lower levels of reticulocyte mRNA_β found when there has been no transfusion with respect to post-transfusal conditions, when β-globin synthesis occurs. It has been shown that high levels of untranslated mRNA_β are present in β₀-thalassaemia among Americans of Chinese descent²³, while some δβ₀-thalassaemias and hereditary persistence of foetal haemoglobin are caused by gene deletion^{17,24-25}. There are many cases of β₀-thalassaemia where the gene for β globin is present but mRNA_β is absent (ref. 5 and P. Tolstoshev and S.O., unpublished observations).

B. Forget (personal communication) has reported similar findings recently, using RNA extracted from samples of blood from one of the patients described here. Ramirez *et al.*²⁶ have reported the isolation of mRNA from Ferrara thalassaemic blood which hybridises incompletely with cDNA_β and have postulated a structurally abnormal mRNA_β which does not hybridise to completion. Our results do not agree with theirs.

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5'-Terminal structure and mRNA stability

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Reovirus mRNAs with 5'-terminal m⁷GpppG^m or GpppG are more stable than mRNA containing unblocked ppG 5'-ends when injected into Xenopus laevis oocytes or incubated in cell-free protein synthesising extracts of wheat germ and mouse L cells. The greater stability of mRNA with blocked 5' termini is not dependent upon translation but seems to result from protection against 5'-exonucleolytic degradation.

Most eukaryotic mRNAs including reovirus mRNA contain a blocked, methylated 5'-terminal 'cap', m⁷G(5')ppp(5')X (ref. 1). The presence of this structure is required for efficient translation of reovirus mRNA in a wheat germ protein synthesising extract². It also facilitates the function of other viral and cellular mRNAs in cell-free systems derived from animal and plant cells³⁻⁷. The cap acts at the level of initiation¹, apparently by promoting the formation of ribonuclease-resistant, stable complexes between mRNA and ribosomes^{8,9}.

It was of interest to determine whether the stability of mRNA is also affected by its 5'-terminal structure. Several eukaryotic mRNAs were previously found to be more stable after microinjection into *Xenopus laevis* oocytes than ribosomal RNAs¹⁰ which have phosphorylated, unblocked 5' ends. Capped mRNAs can be 'unblocked' by β -elimination, that is, chemical removal of the 5'-terminal m⁷G. Although this procedure decreases the translation of treated mRNA, it also alters the 3' terminus and possibly internal sites in the RNA chains. In an attempt to avoid these complications in studies on the role of the cap in eukaryotic protein synthesis, we established conditions for the selective *in vitro* synthesis of reovirus mRNA with 5'-termini of three different types: unblocked (ppG), blocked (GpppG) and capped (m⁷GpppG^m) (ref. 11). We report here that reovirus mRNAs with blocked or capped 5' termini are more stable than unblocked or β -eliminated viral mRNAs in *X. laevis* oocytes and protein synthesising extracts of wheat germ and L cells. In reticulocyte lysates, however, the mRNAs are similarly stable. In addition, the results suggest that unblocked mRNA is degraded exonucleolytically from the 5'-end.

Increased stability of reovirus mRNA containing blocked 5' termini

Radioactive reovirus mRNAs were synthesised in the presence of α -³²P-UTP under previously specified conditions¹¹ designed to obtain molecules with predominately (>93%) one type of 5'-end. In addition to the three types noted above, viral mRNAs with 5'-terminal pppG and pppG^m were prepared by β -elimination of blocked and capped mRNAs, respectively. For stability measurements, ³²P-labelled samples were microinjected into *X. laevis* oocytes¹⁰. At the indicated time intervals, the oocytes were homogenised and the lysates analysed for total and acid-precipitable radioactivity. mRNAs with blocked 5' termini were apparently more stable than those with unblocked, phosphorylated 5' ends (Fig. 1). For example, 8 h after injection of mRNAs containing GpppG or m⁷GpppG^m, ~55% of the intracellular radioactivity remained acid-precipitable. By contrast, values of 20-25% were obtained for the three mRNA samples with unblocked 5' termini, that is, ppG, pppG and pppG^m.

Radioactive material was re-isolated from oocytes at intervals after injection of ³²P-labelled mRNA and analysed by sedimenta-

tion velocity centrifugation. At each time the acid-precipitable radioactivity was present mostly in full-length viral mRNA rather than RNA fragments. Sedimentation profiles of the untreated samples obtained before injection into oocytes included the large (l), medium (m), and small (s) size classes of

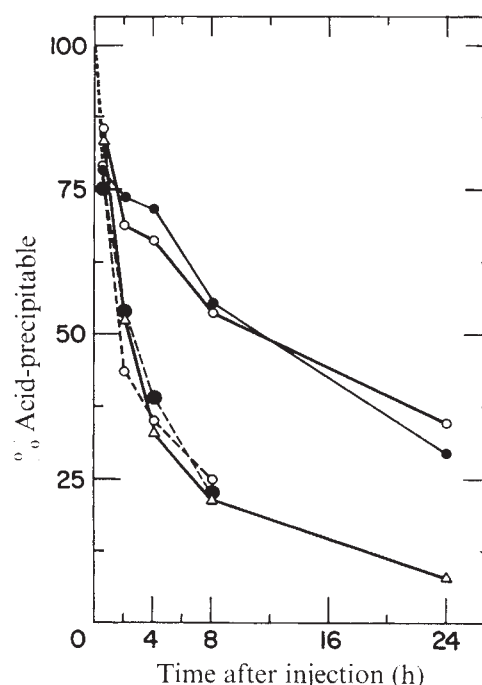


Fig. 1 Degradation of reovirus mRNA injected into *X. laevis* oocytes. Reovirus mRNA was synthesised *in vitro* by the virion-associated RNA polymerase in the presence of α -³²P-UTP in conditions¹¹ that yielded ³²P-labelled products (final specific activity $\sim 1.2 \times 10^6$ c.p.m. per μ g RNA) containing 5'-terminal 94% ppG (with 6% GpppG), 93% GpppG (with 7% ppG), or >94% m⁷GpppG^m. Aliquots of the last two samples were oxidised with periodate and treated with aniline to obtain mRNA with 5'-terminal pppG and pppG^m (ref. 21). The β -elimination was >90% complete as monitored with ³H-methyl-labelled mRNA. RNAs were purified by Sephadex G-100 gel filtration, passed through sterile 0.22- μ m filters, reprecipitated with ethanol and dissolved in H₂O at concentrations of 3.3 μ g μ l⁻¹. For each time point, 10 oocytes were injected with 50 nl of RNA solution per oocyte and incubated at room temperature in 5 ml of amphibian media consisting of 15 mM Tris buffer pH 7.4, 88 mM NaCl, 1.3 mM KCl, 0.7 mM CaCl₂ and 0.8 mM MgCl₂ as suggested by Dr L. Burzio, Rockefeller University. At the indicated times, oocytes were removed, washed three times with 2 ml of media, resuspended in 0.2 ml of 50 mM Tris-HCl, pH 7.6 containing 0.1 M NaCl, 5 mM EDTA, and 0.5% sodium dodecyl sulphate and homogenised in a Dounce homogeniser. The lysates were extracted with an equal volume of redistilled, H₂O-saturated phenol, centrifuged (3000 r.p.m., 10 min), and the aqueous layers were assayed for total and acid-precipitable radioactivity by counting aliquots directly in Aquasol and on Millipore filters after precipitation in 5% trichloroacetic acid. Because the recovery of intracellular radioactivity varied (23-79% of the estimated amount of micro-injected mRNA) presumably due to leakage, values were normalised by expressing them as a percentage of recovered c.p.m. that were acid-precipitable. mRNAs with 5'-terminal m⁷GpppG^m (●), GpppG (○), ppG (▲), pppG^m (●-●) and pppG (○-○).

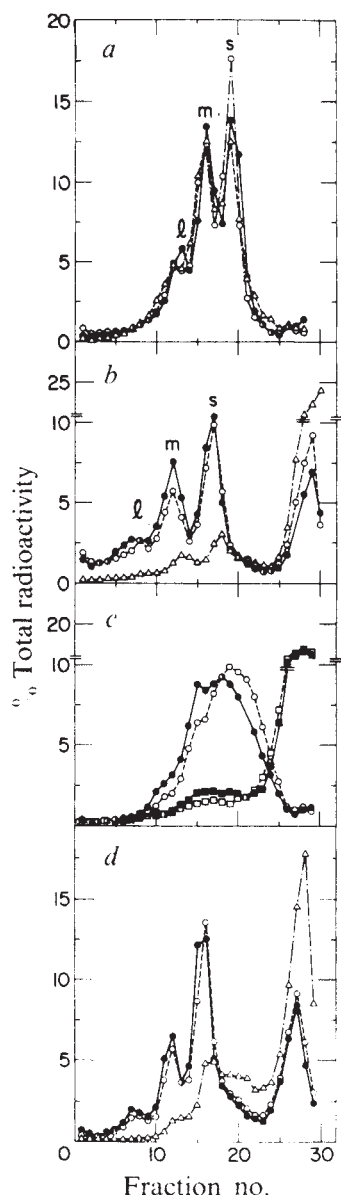


Fig. 2 Sedimentation analysis of reovirus mRNA before and after incubation in oocytes or wheat germ extract. *a*, mRNA synthesised in the presence of α - 32 P-UTP was purified by gel filtration and sedimented in 5–30% glycerol gradients (20 mM Tris-HCl, pH 7.6, 0.1 M NaCl, 1 mM EDTA; SW41–33,000 r.p.m., 16 h). Fractions of 0.4 ml were collected and counted in Aquasol. *b*, The mRNAs containing different types of 5'-ends were injected into oocytes, reisolated after 8 h and analysed as in (*a*). *c*, Blocked and capped 5'-ends of mRNA were converted to 5'-pppG and pppG^m, respectively, by β -elimination²¹. The treated mRNAs were sedimented before (pppG^m, ●; pppG, ○), and after incubation (pppG^m, ■; pppG, □) for 8 h in oocytes. *d*, mRNAs synthesised with α - 32 P-UTP (1 μ g, 46 – 55×10^3 c.p.m.) were incubated in wheat germ extract for 10 min at 26 °C. Radioactivity was then recovered by phenol extraction and analysed as in (*a*). For panels *a*, *b* and *d*, mRNAs with 5'-terminal m⁷GpppG^m (●); GpppG (○); ppG (□).

reovirus mRNA and no slowly sedimenting material (Fig. 2*a*). Following incubation there was a progressive reduction in the proportion of radioactivity in viral mRNA and a corresponding increase in low molecular weight material which was acid-soluble. At all times, however, the fraction of radioactivity sedimenting as intact mRNA was greater for the samples that contained blocked as compared to unblocked 5' termini. This can be seen in the sedimentation patterns of samples extracted 8 h after injection (Fig. 2*b*). Sixty per cent of the radioactivity in the mRNAs with 5'-terminal m⁷GpppG^m or GpppG sedi-

mented in the l, m and s positions while less than 20% of the mRNA containing 5'-ppG remained undegraded. Similar results were obtained with β -eliminated mRNAs containing pppG^m and pppG at the 5'-ends (Fig. 2*c*). Although the chemically-treated RNAs were not resolved into the three size classes, possibly because some chains were nicked during β -elimination, the preparations before injection contained very little radioactivity sedimenting at the top of the gradient. By 8 h after injection, more than 75% of both RNAs had been degraded to slowly sedimenting material. Since mRNAs terminating in pppG^m and ppG showed similar rates and patterns of degradation (Figs 1, 2*b* and *c*), an increase in the number of 5'-phosphates and 2'-O-methylation of the 5'-terminal residue did not stabilise the mRNA.

Degradation of the various mRNAs in each case resulted in low molecular weight products and no skewing of the mRNA peaks, suggesting exo- rather than endonucleolytic cleavage. This effect can be seen most clearly with mRNA that had not been chemically treated (Fig. 2*b*). Allende *et al.*¹⁰ also observed that the stable fraction of *Dictyostelium* mRNA extracted from *Xenopus* oocytes 24 h after injection cosedimented with uninjected marker mRNA. Our findings that the 5'-unblocked

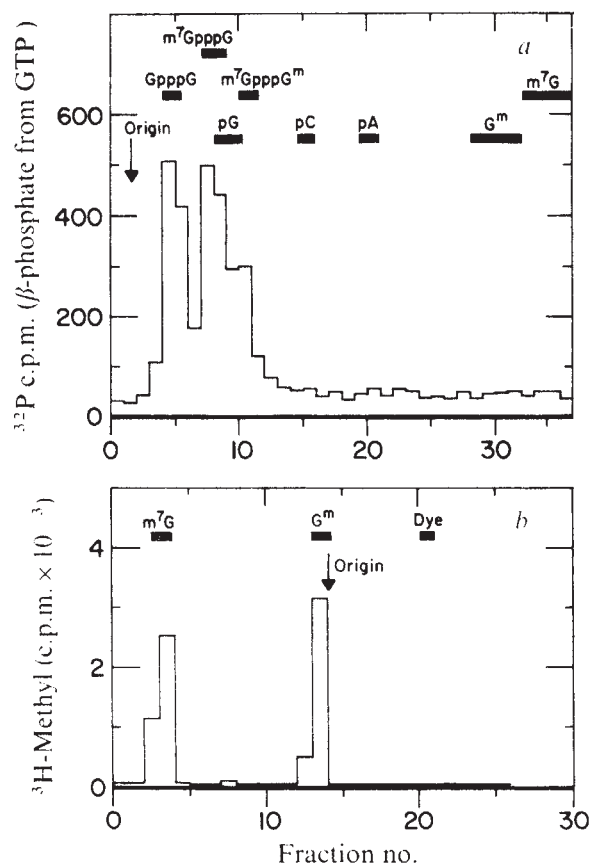


Fig. 3 Analysis of 5'-terminally labelled mRNA after incubation in oocytes. *a*, Reovirus mRNA was synthesised *in vitro* in the presence of β , γ - 32 P-GTP. 5'-adenosylhomocysteine and inorganic pyrophosphatase to obtain products with 5'-terminal Gppp**p*G (ref. 11). The purified mRNA was microinjected (a total of 1 μ g and 16,000 c.p.m. into 50 oocytes), incubated for 9 h, re-extracted, digested with P₁ nuclease and alkaline phosphatase and analysed by paper chromatography in isobutyric acid–0.5 M NH₄OH (10/6 v/v)¹². *b*, 3 H-methyl-labelled mRNA containing 5'-terminal m⁷GpppG^m was injected (50 oocytes each with 50 nl, 0.08 μ g, 1,200 c.p.m.) and after 24 h was re-isolated, digested with P₁ nuclease and phosphatase and analysed by high voltage paper electrophoresis at pH 3.5 (ref. 12). The enzyme-resistant, 3 H-labelled blocked structures migrating as a peak close to pG were eluted, digested with nucleotide pyrophosphatase and phosphatase and re-analysed by electrophoresis (60 V cm⁻¹, 45 min). Marker compounds were located under ultraviolet light, and 1-cm strips were cut and counted in toluene-based scintillant¹².

mRNAs are less stable than blocked mRNAs, together with the earlier report¹⁰, suggest that mRNA degradation in oocytes occurs from the 5'-end by an exonucleolytic, processive mechanism.

Studies of the fate of eukaryotic mRNAs in *X. laevis* oocytes showed that puromycin treatment decreased stability, and it was proposed that association with ribosomes affords some protection of mRNA against nucleolytic degradation¹⁰. Because methylation of reovirus mRNA promotes initiation complex formation *in vitro*¹⁻⁸, we tested whether the 5'-terminal GpppG in injected viral mRNA was converted to m⁷GpppG^m by methyl transferase(s) in oocytes. mRNA was synthesised with β - γ -³²P-GTP as radioactive precursor in order to label specifically the blocked 5'-terminal structure, GpppG (refs 11, 12). In the synthetic conditions used¹¹, the mRNA contained 5'-GpppG (93%) and small (7%) amounts of 5'-p⁺pG. The RNA was injected into oocytes, recovered after 9 h and analysed as described in the legend to Fig. 3 to identify the 5'-terminal structure. About 40% of the ³²P-labelled, blocked 5'-termini remained unmethylated and migrated in the position of GpppG (Fig. 3a). The rest of the GpppG 5'-ends were methylated, however, and enzyme treatment of the mRNA yielded a mixture of m⁷GpppG and m⁷GpppG^m. We conclude that *X. laevis* oocytes, like protein synthesising extracts from plant and animal cells^{2,4-7}, contain enzyme activities that methylate the 5'-end of reovirus mRNA and presumably other mRNAs as well. Because the blocked reovirus mRNA in oocytes was only partially methylated at the 5'-end (~60% conversion of GpppG to m⁷GpppG^m at 9 hr), this event cannot account for stability since the degradation kinetics of unmethylated, blocked mRNA and capped mRNA are almost identical during the 24 h after microinjection and both mRNAs are equally more stable than unblocked mRNA (Fig. 1). Consistent with this conclusion, blocked mRNA was also more stable than unblocked mRNA in wheat germ protein synthesising extracts in conditions that prevented methylation.

In order to determine if caps in mRNA turn over, reovirus mRNA synthesised in the presence of ³H-methyl-S-adenosylmethionine and labelled exclusively in the 5'-terminal m⁷GpppG^m was injected into oocytes. After 24 h, the RNA was reisolated, digested with P₁ nuclease and phosphatase, and analysed by paper electrophoresis¹². Over 90% of the radioactivity migrated in the position of blocked structures (data not shown). This material was eluted, digested with nucleotide pyrophosphatase and alkaline phosphatase, and re-analysed by electrophoresis (Fig. 3b). Radioactivity was equally distributed between two ³H-methyl-labelled components migrating in the positions of 7-methylguanosine (m⁷G = 3,680 c.p.m.) and 2'-O-methylguanosine (G^m = 3,690 c.p.m.). Thus, both methylated residues in caps were conserved and the 5'-terminal m⁷G did not turn over following injection of reovirus mRNA into *X. laevis* oocytes. Although an enzyme activity that cleaves m⁷G-containing cap structures has been detected in animal cell extracts, it did not hydrolyse caps in intact mRNA¹³. A novel phosphodiesterase that cleaves pyrophosphate linkages in mRNA has been purified from tobacco cells¹⁴. In our studies, however, there was no evidence for differential turnover of caps when reovirus ³H-methyl-labelled mRNA was incubated for 90 min in conditions of protein synthesis in wheat germ extract and then analysed as in Fig. 3b (unpublished results). As reported for cellular mRNAs in mouse kidney¹⁵ and L cells¹⁶, caps in reovirus mRNA apparently decay with the mRNA chain when incubated in *X. laevis* oocytes or wheat germ extract.

Reovirus mRNA degradation independent of translation in wheat germ extract

Reovirus mRNAs with blocked or capped 5'-termini are more stable than unblocked mRNAs in oocytes, consistent with 5'-exonucleolytic degradation. Although *X. laevis* oocytes are useful for *in vivo* studies on mRNA stability and function^{10,17}, it is not yet known if reovirus mRNAs are translated in this system. To investigate the relationship between translation and

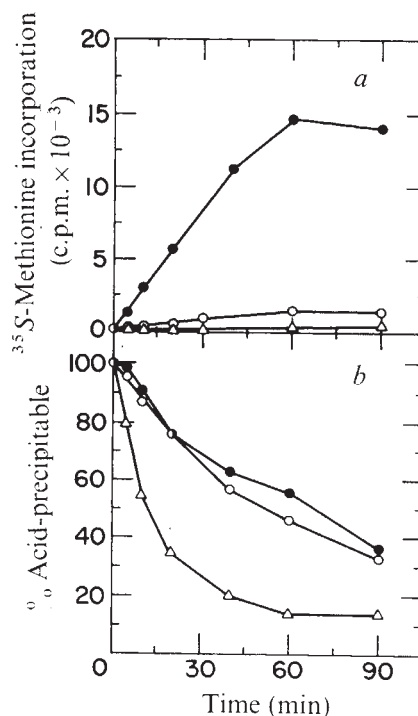


Fig. 4 Translation and degradation of reovirus mRNAs in wheat germ extract in the presence of the methylation inhibitor, S-adenosylhomocysteine (Adohcy). *a*, For protein synthesis the incubation mixture (50 μ l) contained 12 mM HEPES buffer (pH 7.3), 3 mM Mg acetate, 1 mM ATP, 0.05 mM GTP, 5 mM creatine phosphate, 10 μ g creatine phosphate kinase, 75 mM KCl, 2 mM dithiothreitol, 100 μ M Adohcy, ³⁵S-methionine (10 μ Ci, 250 Ci mol⁻¹), 0.01 mM of each of the other 19 amino acids, 1 μ g reovirus mRNA, and wheat germ extract equivalent to 215 μ g protein prepared as before². Incubation was at 26 °C, and 5 μ l aliquots were collected in 0.2 ml 5% trichloroacetic acid, heated for 10 min at 90 °C, and the acid-precipitable radioactivity collected on Millipore filters was counted in toluene-based scintillant. *b*, Degradation was measured in the same reaction conditions, except that ³⁵S-methionine was replaced by 0.01 mM non-radioactive methionine and ³²P-labelled reovirus mRNAs (1 μ g, 110–130 $\times$ 10³ c.p.m.) prepared with α -³²P-UTP were used. Incubation was at 26 °C; 5- μ l aliquots were extracted and radioactivity precipitated in 5% trichloroacetic acid was measured as described in (*a*). mRNAs contained 5'-terminal m⁷GpppG^m (●), GpppG (○) and ppG (△).

the stabilities of mRNAs with variable 5'-terminal structures, studies were performed using wheat germ protein synthesising extract which was shown previously to translate reovirus mRNA containing 5'-terminal m⁷GpppG^m (ref. 2). The same viral mRNA with 5'-terminal GpppG or ppG functions poorly or not at all in protein synthesis when the conversion of GpppG ends to m⁷GpppG by wheat germ endogenous methyl transferase(s) is prevented by addition of S-adenosylhomocysteine². While the presence of this methylation inhibitor blocks translation of unmethylated reovirus mRNA in wheat germ extract, it does not affect protein synthesis directed by mRNA that is pre-methylated during transcription. As found before, and shown in Fig. 4a, only capped mRNA was efficiently translated in the presence of S-adenosylhomocysteine. Under the same conditions, the relative mRNA stabilities in wheat germ extract were similar to those found with microinjected oocytes, that is, the capped and blocked mRNAs were both more stable than unblocked mRNA (Fig. 4b). Since capped mRNA (that is, blocked and methylated) forms stable complexes with wheat germ ribosomes while blocked but unmethylated mRNA does not⁸, the similar stability observed for these two types of mRNAs is not due to protection by association with ribosomes. Thus, increased stability seems to be an intrinsic property of mRNAs with blocked 5'-terminal structures. From the results shown in Figs 4a and b, it seems that 5'-blocking without methylation is sufficient to enhance reovirus

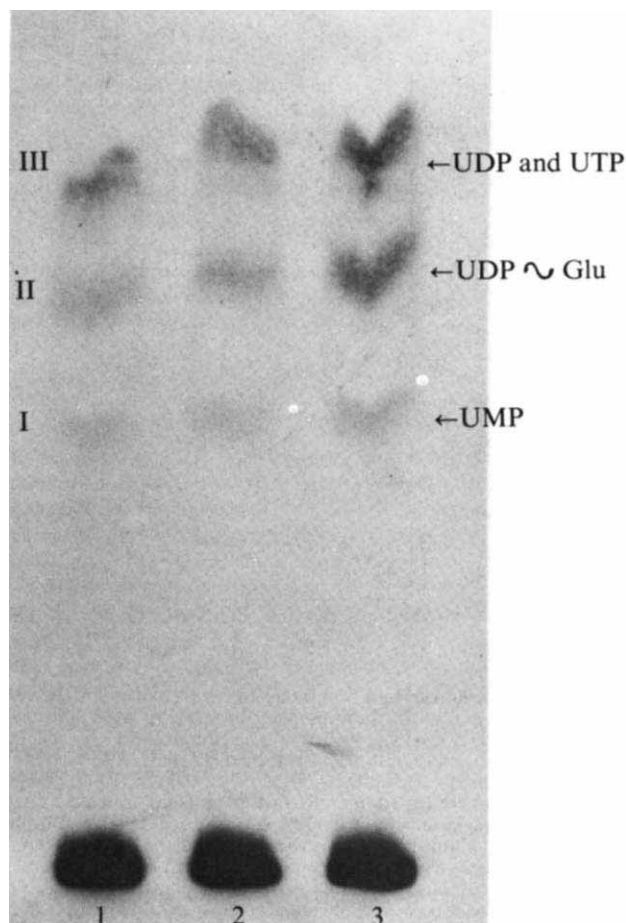


Fig. 5 Analysis of degradation products of reovirus mRNA. Reovirus mRNAs labelled internally with α - ^{32}P -UMP were incubated for 10 min in wheat germ extract in the conditions described for Fig. 4b. Radioactive materials were recovered from reaction mixtures by phenol extraction and analysed by high voltage paper electrophoresis (50 V cm^{-1} , 50 min) in 10% pyridine-acetate buffer (pH 3.5) with marker compounds. Total radioactivity shown in the autoradiogram in tracks 1, 2 and 3 was 110 , 120 , and 108×10^3 c.p.m., respectively. The distribution of radioactivity in each track determined by direct counting of each spot after cutting the paper, was: track 1—origin 87%, band I, 1.7%; band II, 5% and band III, 6.3%. Track 2—origin 83%; band I, 2%; band II, 6.6% and band III, 8.4%. Track 3—origin 60%; band I, 5%; band II, 15% and band III, 20%. Reovirus mRNAs in tracks 1, 2 and 3 contained 5'-terminal m⁷GpppG^m, GpppG, and ppG, respectively. UDP ~Glu is an abbreviation for uridine diphosphate glucose.

mRNA stability, but translation in wheat germ extract depends on the presence of the methylated blocked structures, m⁷GpppG^m. The more rapid degradation of unblocked reovirus mRNAs, which have the same sequence and size as blocked or capped mRNA but a different 5'-terminal structure, suggests that mRNA is degraded exonucleolytically from the 5'-end.

5'-Exonuclease cleavage of unblocked mRNA

Reovirus unblocked mRNA was less stable than 5'-blocked mRNA in microinjected oocytes or in wheat germ cell-free extract (Figs 1, 4b). When unblocked radioactive mRNA was re-extracted after incubation for 8 h in oocytes, the radioisotope was present in full-size mRNA and in low molecular weight degradation products (Fig. 2b). The same results were obtained when mRNA was incubated in wheat germ extract for 10 min and subsequently analysed by velocity sedimentation in a glycerol gradient (Fig. 2d). Little radioactivity was observed in products of intermediate size and most of the degraded material sedimented at the top of the gradient. The low molecular weight products were characterised by paper electrophoresis

(Fig. 5). Radioactive material analysed after incubation of ^{32}P -labelled mRNA in wheat germ included the mRNA at the origin and three fast-migrating bands but no intermediate spots in the position of oligonucleotides, CMP, AMP, or GMP. The compound in band I corresponds to 5'-UMP. Band III was eluted and identified as a mixture of UDP (32%) and UTP (68%) by paper chromatography with marker compounds in isobutyric acid–0.5 M NH_4OH (10:6 v/v). The component in band II migrated in the position of UDP-glucose or UDP-galactose and the ^{32}P -phosphate present in this material was resistant to hydrolysis by alkaline phosphatase. We have not identified the compound(s) in band II, but it has a net charge of -2 by DEAE-cellulose chromatography and can be enzymatically synthesised from ^3H -UTP in wheat germ extract, consistent with it being a UDP-sugar derivative. In addition, there is a clear precursor-product relationship between the 5'-UMP (band I), the UDP and UTP (band III) and the presumptive UDP-sugar (band II). When ^3H -labelled 5'-UMP (360 pmol, 15 μCi) was incubated for 20 min in wheat germ extract, 54% of the input radioactivity was incorporated into the presumptive UDP-sugar compound and 29% into a mixture of UDP and UTP. By contrast, the presumptive UDP-sugar yielded no 5'-UMP, UDP, or UTP on incubation in wheat germ extract. Thus, the primary degradation product of mRNA labelled with α - ^{32}P -UTP was 5'-UMP which in wheat germ was further

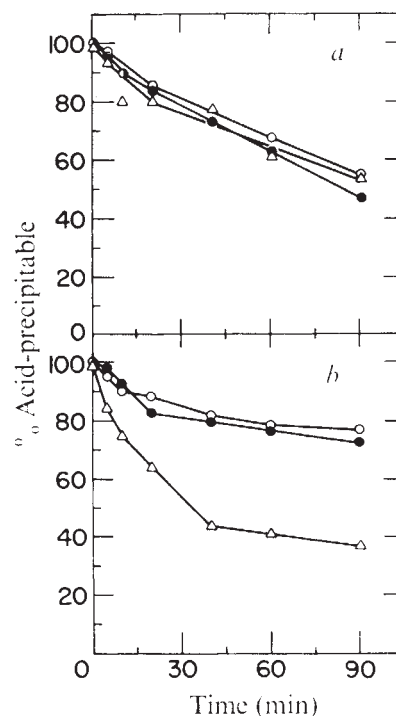


Fig. 6 Test for exonuclease activity in rabbit reticulocyte lysate and mouse L cell extract. Reovirus mRNAs labelled internally with α - ^{32}P -UMP ($20 \mu\text{g ml}^{-1}$, 100 – 120×10^3 c.p.m.) were incubated at 26°C in conditions used for *in vitro* protein synthesis. *a*, Reticulocyte lysate: the reaction mixture ($50 \mu\text{l}$) contained 12 mM HEPES buffer (pH 7.5), 75 mM KCl, 1.5 mM Mg acetate, 0.8 mM ATP, 0.16 mM GTP, 100 μM Adohey, 10 mM creatine phosphate, 11 μg creatine phosphate kinase, 5 mM dithiothreitol, 0.1 mM each of 20 amino acids, 20 $\mu\text{g ml}^{-1}$ haemin, 1 μg ^{32}P -labelled reovirus mRNA and lysate equivalent to 2.4 mg protein (provided by W. F. Anderson). *b*, L cell protein synthesising extract: the mixture contained 20 mM HEPES (pH 7.6), 120 mM KCl, 5 mM Mg acetate, 6 mM β -mercaptoethanol, 1 mM ATP, 0.2 mM GTP, 100 μM Adohey, 0.1 mM each of 20 amino acids, 5 mM dithiothreitol, 10 mM creatine phosphate, 10 μg creatine phosphate kinase, and L cell extract equivalent to 252 μg protein (provided by G. Koch). Acid-precipitable radioactivity was assayed as for Fig. 4b. mRNAs contained 5'-terminal m⁷GpppG^m (●), GpppG (○) and ppG (△).

metabolised by the route $UMP \rightarrow UDP \rightarrow UTP \rightarrow UDP\text{-sugar}$. The reovirus mRNA degradation products from *X. laevis* oocytes yielded the same four uridine derivatives when subjected to electrophoresis, and the primary mRNA degradation products in oocytes also are probably 5'-mononucleotides. These observations suggest that the exonuclease processively cleaves unblocked mRNA in a 5' to 3' direction yielding 5'-mononucleotides. Confirmation of these characteristics will require studies of the purified enzyme(s).

Like *Xenopus* oocytes and wheat germ extract, L cell protein synthesising extract also contains an enzyme activity that degrades unblocked reovirus mRNA more rapidly than blocked or capped mRNA (Fig. 6b). In contrast, we did not detect a differential breakdown of unblocked mRNA in reticulocyte lysate incubated at a 10-fold higher ratio of protein to mRNA than used for L cell extracts (Fig. 6a). It will be of interest to determine whether mRNA degrading activities with similar properties exist generally in eukaryotic cells and if so, whether they are involved in mRNA turnover and regulation of cell division and differentiation.

Discussion

In this report, reovirus mRNAs with different types of 5'-termini were used to demonstrate that, in addition to a role in the initiation of protein synthesis, cap structures have an important stabilising effect on mRNA. In accordance with this function, and consistent with the lower stability of unblocked reovirus mRNA in *Xenopus laevis* oocytes and extracts of wheat germ and L cells, a nuclease activity which apparently cleaves unblocked RNA exonucleolytically and probably processively was detected in the amphibian, plant, and mammalian systems. Rabbit reticulocyte lysate was an exception and contained little or no nuclease activity of this type. Assuming that the nuclease activity has a role in mRNA turnover, a highly differentiated cell such as the reticulocyte which has lost its transcriptional machinery might be expected to have no such activity.

In several previous studies, the β -elimination reaction was used to remove m⁷G from capped mRNA in order to test its influence on translation *in vitro*. The destabilising effect of unblocking (Figs 1, 2c) was then unknown, however. Shih *et al.*³ compared the efficiency of translation of m⁷G-capped and β -eliminated brome mosaic virus RNA and demonstrated that the positive effect of m⁷G cap on translation in wheat germ extract was more pronounced at low RNA concentrations ($< 20 \mu\text{g ml}^{-1}$) than at high levels ($100 \mu\text{g ml}^{-1}$) of RNA. The difference in the translational efficiencies might be due to degradation of some of the β -eliminated RNA by wheat germ exonuclease activity. Smith *et al.* and Baglioni *et al.* recently found that the extent of dependence on m⁷G for mRNA translation varies with the concentration of potassium ions in protein synthesising reaction mixtures (personal communications). Therefore, in order to understand better the role of the cap structure in the translation of various RNAs, future experiments should be designed to take into account cellular exonuclease(s)

and other factors that affect translation in cell extracts.

Polio, encephalomyocarditis (EMC) and satellite tobacco necrosis virus (STNV) RNAs do not contain 5'-cap structures¹. These RNAs, however, are sufficiently stable to be translated in various model systems and also function as viral messengers in infected cells. How are these uncapped mRNAs stabilised? Allende *et al.*¹⁰ reported that purified ribosomal RNA and 5S RNA are rapidly degraded when microinjected into *Xenopus* oocytes, but globin and *D. discoideum* mRNAs, RNA in ribosomes, and *Escherichia coli* suppressor 3 tRNA^{tyr}, which cannot be aminoacylated in oocytes, are all stable. These observations can be explained if extensive and specific secondary structures or the association of proteins, as observed for tRNA, and ribosomes, respectively, prevent 5'-cleavage by exonuclease activity. In this regard, it was found recently that polio virion RNA, the first viral messenger in polio-infected cells, has a viral protein covalently linked at the 5'-end¹⁸. Poliovirus RNA, EMC virus RNA, polio polysomal RNA, STNV RNA and possibly other messenger RNAs that lack a 5'-5'-inverted structure at the 5'-end may be protected against exonuclease attack by covalent or non-covalent interaction with protein(s) or by 5'-terminal secondary structure. In conclusion, the presence of a blocked or capped 5'-end results in protection of RNA against 5'-exonucleolytic degradation. In some RNAs, for example avian sarcoma virus (ASV) genome RNA which is capped¹⁹ but translated poorly *in vitro*²⁰, caps may function mainly to confer stability rather than, as with reovirus mRNA, to promote translation. In support of this suggestion, the cap on ASV 35S RNA is not protected against RNase T1 digestion by mouse ascites 40S ribosomal subunits (M. Kozak, unpublished).

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letters to nature

Radio counterpart of the X-ray source 3U0901-09

A RECENTLY published position¹ of the X-ray source 3U0901-09 obtained from the Ariel V experiment overlaps the extent of the Abell cluster A754. Previous radio continuum observations of this area² revealed a complicated radio emission distribution.

We are mapping a sample of Abell clusters and have made a map of the area containing A754. We find that the position ellipse of the X-ray source is in good agreement with a radio source complex with a very steep spectrum.

The observations were made at 2,695 MHz from the secondary focus of the 100-m radiotelescope of the Max-Planck-Institut für Radioastronomie. The beamwidth at this frequency is

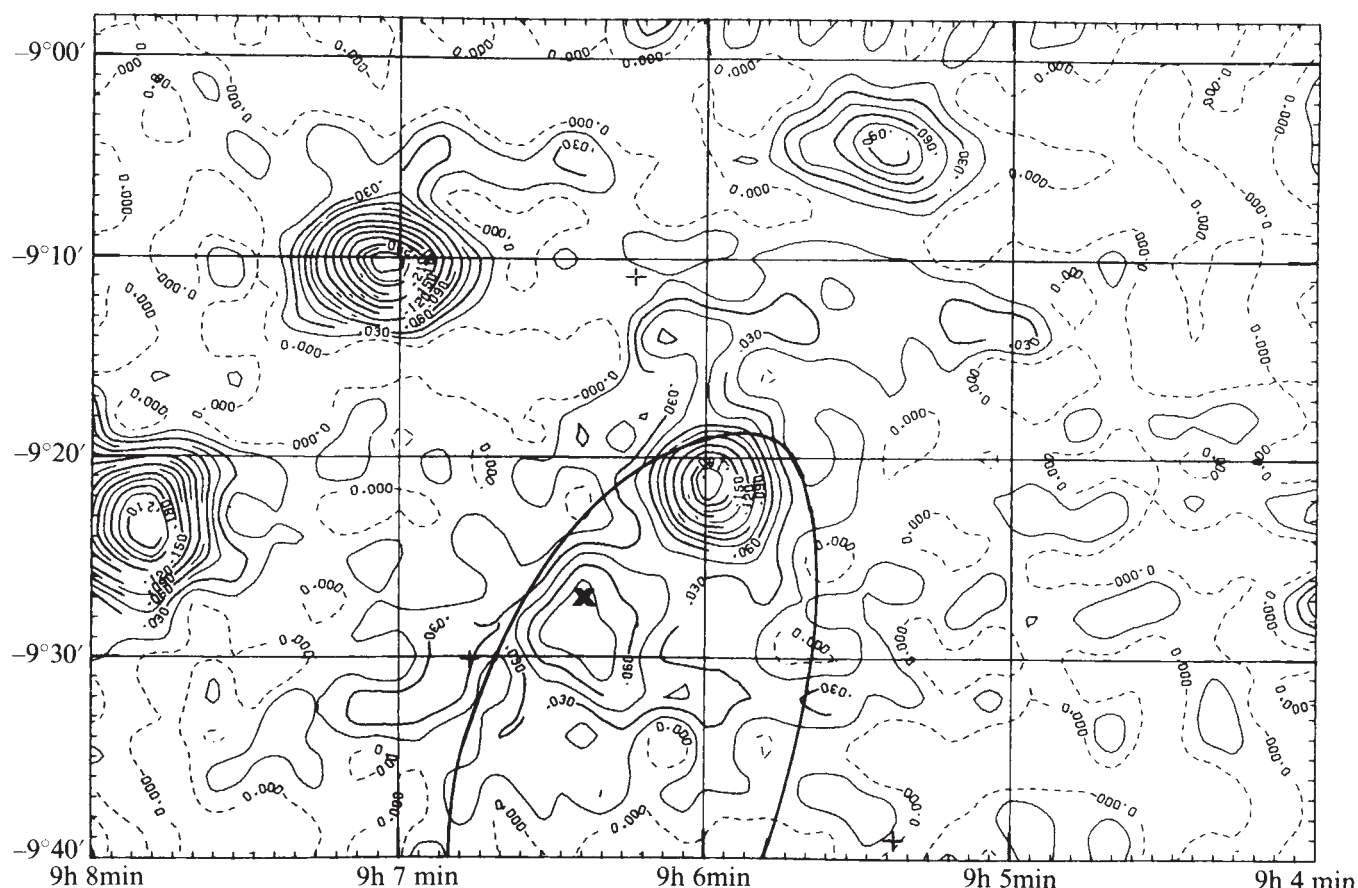


Fig. 1 The 11.1-cm map at an area near A754. Contours are in steps of 15 mJy per beam. Dashed contour shows the zero level. X, centre of A754. The position ellipse of the Ariel V measurement is also shown. Note 1 Jy per beam = $1.5 \text{ KT}_a \sim 2.0 \text{ KT}_{mb}$.

4.4 to 3 dB points. The receiving system is a dual channel correlation radiometer. In this receiving mode a single circular polarisation is combined with the signal from a liquid nitrogen termination in a hybrid, amplified in two phase-stable uncooled parametric amplifiers and correlated at intermediate frequency. The output signal is the difference in power between one circular polarisation and the cold load. The system noise temperature is about 100 K. The bandwidth over which excellent phase stability and tolerable low level of interference are possible is 100 MHz. With this receiver performance fluctuations of 14 mK r.m.s. s^{-1} are expected. In fact, some degradation of performance due to low-level interference and atmospheric fluctuations takes place, requiring an integration of 10 s to achieve 5 mK r.m.s. noise.

Observations were made on 25 April 1976. Scanning of the $42' \times 60'$ area was in right ascension at $60' \text{ min}^{-1}$. Scans were made at intervals of $1'$ in declination, requiring 43 steps for the whole map. Two coverages were made and the intensity arrays added to give the map (Fig. 1). Calibration used the source 3C 286 ($S_{1.1} = 10.3 \text{ Jy}$) and an internal switched noise diode.

Figure 1 shows four sources, three of which are unresolved by our beam. Extended emission is seen within the position ellipse given for 3U0901-09 by the Ariel V sky survey instrument¹. The position ellipse also contains the centre of the Abell cluster A754. Previous observations of A754 were reported by Owen² at 1,420 MHz with the 300-ft NRAO radiotelescope. Since his beamwidth was $10'$ only one extended source was found at 1,420 MHz in a position which agrees with the centre of gravity of the point source seen at 11 cm in the uppermost end of the position ellipse and the extended source coincident with A754. We have determined the flux of all sources at 11 cm wavelength and have collected our flux values together with Owen's values in Table 1. Our errors in flux determination were

estimated to be $\pm 10\%$. We estimate the error in the 1,420 MHz flux to be $\pm 20\%$ for the point sources and $\sim 30\%$ for the extended one. The spectral index of the two point sources (Nos 3 and 4 in Table 1) is in the range typical for nonthermal radio sources. The spectral index α ($S \propto \nu^{-\alpha}$) of 2.5 ± 0.8 found for the complex which includes the flux of the sources 2a and 2b makes this source similar to other X-ray radio sources or sources in galaxy cluster^{3,4,5}. The total flux of the whole complex may be higher than that given in Table 1 due to further emission outside the contours within which we integrate, but it should correspond to the flux which was determined, but it should correspond to the flux which was determined by Owen at 1,420

Table 1 Flux of sources at 11 cm wavelength, and spectral index, α

Source	RA (1950.0)	Dec. (1950.0)	Flux density (mJy) 1420 MHz	2695 MHz	Spectral index α
1	09h05min25.5	-09 04.3'	---	100	2.5 ± 0.8
2a	09 05 57.6	-09 21.6	1510	190	
2b	09 06 24.0	-09 27.0		100	
3	09 07 02.4	-09 10.5	330	285	
4	09 07 49.1	-09 24.0	440	255	0.8 ± 0.5

MHz. In view of this unusually steep spectrum we propose that the X-ray emission comes from this extended radio complex. We have no knowledge of the temporal variations of this source, which may be one of the reasons for this steep spectral index, and we suggest that observations at a number of frequencies should be made over a period of time to finally delineate the spectral index.

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Spectroscopic evidence for interstellar grain clumps in meteoritic inclusions

THE occurrence of a primitive, presolar grain component in carbonaceous chondrites has been indicated by several independent lines of evidence, including the discovery of isotopic abundance anomalies^{1,2}. The occurrence of organic molecules, including amino acids, in these meteorites is also known^{3,4}, but their connection with a genuinely pre-solar grain component has been conjectural. We show here that an extract of organic material from the Murchison carbonaceous chondrite has an ultraviolet spectrum with an absorption band centred at $\lambda \approx 2,200$ Å. The similarity of this feature with the well-known interstellar absorption band at the same wavelength, gives strong credence to an interstellar grain component within this meteorite.

Carbonaceous chondrites contain a significant mass fraction of organic compounds, mainly in the form of aromatic polymers. Typically about 30% of this material is of relatively low molecular weight and could be extracted by using hexafluoroisopropanol as a solvent. The remaining 70% is of higher molecular weight and is usually insoluble⁵.

A sample of the Murchison chondrite (supplied by Dr. Onuma) was used to obtain an organic molecule extract with hexafluoroisopropanol. The absorption spectrum of this extract (Fig. 1) shows a conspicuous absorption hump centred at $\lambda \approx 2,200$ Å, having a half-width ≈ 300 Å and bearing a remarkably strong similarity to the well-known interstellar extinction feature⁶⁻⁸. A band of this type is a property of a wide class of organic molecules having conjugated double bonds, for example, $C-C-C$, $C-C-C-C$, $C=C-C$, $C-C=O$, with a typical cross-section of $\sim 4 \times 10^{-17}$ cm² (refs 9, 10).

Although the 2,200-Å absorption band in the interstellar extinction curve has usually been attributed to graphite par-

ticles¹¹⁻¹³, this explanation is unsatisfactory. Spherical or nearly spherical graphite particles of radii ~ 150 Å are required to reproduce the observed features of the astronomical band¹⁴. Non-spherical particles, or spherical particles with much larger radii will shift the band centre away from $\lambda 2,200$ Å, or distort the symmetry of the band. An origin of the interstellar $\lambda 2,200$ Å feature in terms of complex organic molecules lodged in grain clumps would have an advantage in that the mean absorption profile (arising from electronic transitions in an ensemble of molecules) has a central wavelength and width which are independent of the shapes and sizes of the host grain clumps. On the basis of this interpretation, the astronomical and meteoritic spectral data would provide further supporting evidence for a connection between pre-solar interstellar material and inclusions in carbonaceous chondrites.

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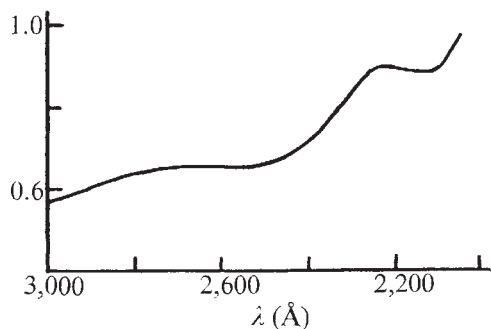
Prebiotic molecules and interstellar grain clumps

INTERSTELLAR molecules detected by radioastronomical techniques in clouds such as OMC 1 and 2 in the Orion Nebula and Sgr B2 in the galactic centre span a wide range of types and complexity. Among the heaviest of the molecules recently discovered is cyanodiacetylene¹ ($H-C \equiv C-C \equiv C-N$). There have been earlier detections of precursors to the simplest known amino acid glycine (formic acid and methanimine), and probable detections of polyoxymethylene polymers and copolymers²⁻⁵ in interstellar clouds. We discuss here a possible identification of organic molecules of even greater complexity, and its implications for the start of biological activity.

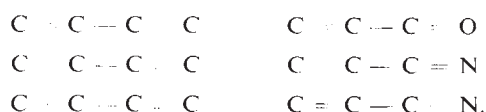
Large departures from thermodynamic equilibrium in the interstellar medium and the co-existence of solid grains, molecules, radicals, ions and ultraviolet photons provide conditions which are ideal for the assembly of 'exotic' molecular species. If clumping of 100 Å-sized dust grains occurred by a process which we have discussed⁶, highly complex organic molecules could be formed and become securely trapped during the 'welding' process of smaller grains. Such grain clumps could be widely dispersed amongst the particulate material in the Galaxy, being responsible for most of extinction and polarisation of starlight.

The spectral identification of highly complex organic species in the interstellar medium might seem hopeless. A property

Fig. 1 The absorption curve of organic material in the Murchison carbonaceous chondrite extracted using hexafluoroisopropanol as a solvent.



common to a wide class of such molecules, however, is an absorption band centred at $\lambda \simeq 2,200 \text{ \AA}$ with a half-width $\sim 250 \text{ \AA}$, which arises mainly due to $\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$ electronic transitions in conjugated multiple bonds such as:



The absorption occurs due to transitions between electrons in n and π -orbital states to antibonding π^* states^{7,8}. Specific examples of such transitions and relevant spectroscopic data have been cited elsewhere⁹. In general, this absorption band occurs in most highly complex organic species, with a typical value of $\sim 200\text{--}300 \text{ \AA}$ for the half-width, and with a molar extinction coefficient $\epsilon \simeq 10^4 \text{ cm}^2 \text{ (g mol}^{-1}\text{)}^{-1}$ (refs 7, 9), which implies an absorption cross-section of $\sim 4 \times 10^{-17} \text{ cm}^2$. A typical case is the curve for mesityl oxide (Fig. 1).

It is tempting to identify this feature with the well-known 2,200- $\text{\AA}$ band of the interstellar extinction curve. The smeared-out mass density ρ of molecules with molecular weight M required to produce the observed extinction coefficient at 2,200 $\text{\AA}$ of $\sim 1.5 \text{ mag kpc}^{-1}$ (refs 10, 11) in the interstellar medium is

$$\rho \simeq 2 \times 10^{-27} (M/100) \text{ g cm}^{-3}.$$

For $M \sim 100$ we obtain a value of ρ which is $\sim 10\%$ of the total available CNO density in the interstellar medium. If this material is assumed to be homogeneously mixed with silicate-type grains of radius a , the condition for optical depth less than unity within a single grain at the band centre is

$$a < \sim 3 \times 10^{-5} \text{ cm}.$$

It may not be fortuitous that this upper limit is about twice the 'typical radius' required for interstellar silicate grains.

Several amino acids of biological importance have been discovered in carbonaceous chondrites¹² and there has also been an identification which is strongly suggestive of the existence of polyoxymethylene polymers or copolymers in the Allende carbonaceous chondrite¹³. Such polymers could have served as glueing agents for smaller grains in prestellar molecular clouds. It is also of interest that an extract of organic

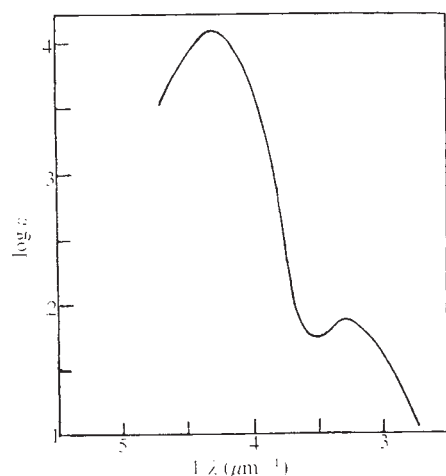
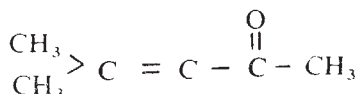


Fig. 1 Molar extinction coefficient $\epsilon \text{ (cm}^2 \text{ (g mol}^{-1}\text{)}^{-1}\text{)}$ of mesityl oxide



as a function of inverse wavelength⁹.

materials from the Murchison chondrite using hexafluoroisopropanol as a solvent showed a 2,200 $\text{\AA}$ absorption feature, typical of conjugated double bonds of the type considered above, and similar to the interstellar extinction hump at this wavelength¹⁴.

We tentatively conclude that these data could be interpreted as independent new chemical evidence of the existence of composite grain clumps in the interstellar medium and in carbonaceous chondrites. Moreover, such grain clumps probably include a significant mass fraction of highly complex organic, prebiotic molecules which could have led to the start and dispersal of biological activity on the Earth and elsewhere in the Galaxy.

Even if all the CNO elements on the Earth's surface were initially in the form of the biologically important 20 amino acids, their linkage into feasible peptide chains could not have occurred by chance. Some process of natural selection is required, and this is usually held to have occurred under terrestrial conditions. If interstellar grain assembly occurred in prestellar molecular clouds already containing these amino acids, pre-Darwinian selection leading to the production of self-replicable peptide chains, could have depended upon the sticking properties of the various molecules on to the polymeric coatings on 100 $\text{\AA}$ -sized grains. A welded composite grain with a 'cellular' structure on the scale of $2\pi \times 100 \text{ \AA}$ could have served as the host system for the earliest primitive gene in a manner similar to that discussed by Cairns-Smith¹⁵.

We now consider the selective mechanisms which could have operated in the growth and evolution of grain clumps, and their possible bearing on the development of a primitive biological system. Not all polymer coatings have the same sticking probabilities α (ref. 6). Some combinations are more probable than others. Suppose α denotes the average sticking probability. What would be the optimum situation for clump growth? A reproducible situation with sticking probability only slightly above α would be better than a non-reproducible situation with probability much greater than α . This is because the latter system, if it occurred, would soon be covered up by further growth of clumps. Once the possibility of a reproducible system is admitted, what reproducible system, among possibly many such systems, would become most widespread? The answer to this question is: a system which also promoted clump division. This leads not only to more clumps but also to a greater total mass of clump material, because of the advantageous surface/volume ratio.

Lastly, we turn to the problem of protection of pre-biotic material against external disruptive agencies, for example, ultraviolet light. Whilst this protection could be provided by an outer shield of solid grains, such a system would be biologically inert. A clump stripped of its outer polymeric coating would not be suitable for sticking, even if it was able to preserve itself. A more refined protection system in the nature of a biological cell wall would be much better, since such an outer surface could permit organic molecules to stick, as well as to diffuse through it.

Thus there seem to be two somewhat different types of selective process which could be operative under interstellar conditions. First, a competition for clump growth in the absence of disruptive agencies, for example, within a pre-stellar cloud; and second, a competition for organic materials (or even for sticking) in a more hostile environment in the presence of disruptive agencies. With the development of a cell wall, the last step could presumably be to 'split out' the inorganic grains which started off the whole process.

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Gravitational radiation detector observations in 1973 and 1974

MARYLAND-ARGONNE gravitational radiation detector observations for 1973-74 have recently been published¹. This letter contains additional information about how the data were taken and analysed.

A primary goal was to eliminate any unconsciously applied human bias which could affect results. To this end, I removed myself from direct participation in the data reduction beginning in 1972. Mr Michael Lee developed programs for a magnetic tape and computer system of data analysis. A number of checks of the computing were set up. The first check was an independent, analogue experiment, in which data were recorded on pen-and-ink charts, with an on-line computer marking the charts whenever the Maryland and Argonne detectors both crossed threshold within 0.1 s. The pen-and-ink recorder experiment had two outputs, one with, and one without, a time delay introduced into one of the channels before the computer looked for simultaneous threshold crossings. Charts for the data with and without the time delay were marked in coded form (which changed from time to time). Mrs Alessandra Exposito searched the charts for statistically significant coincidences without knowing which charts were which. This analogue procedure found excess coincidences at zero time delay during the same periods that the computerised experiment found them.

In a second checking procedure, Mr William Davis inserted artificial pulses into the gravitational radiation detectors at certain times unknown to the programmer, Mr Michael Lee. Lee correctly identified times when artificial pulses were present and absent. Third, Mr Bruce Webster prepared copies of old data tapes, some identical with the originals and others with one or more channels replaced by the same detector data from another time period. Lee had no knowledge of the information recorded on the magnetic tapes. Lee's analysis correctly identified these tapes.

The telephone circuit from the Argonne detector to Maryland was suspect even though the data were transmitted in coded digital form. The recent paper includes all data for a period when there was no telephone circuit and clock-synchronised recorders were employed at the two detector sites. An excess of coincidences over accidentals was observed during that period.

A major effort has been made to eliminate computing errors. Maryland magnetic tape data were sent to several other research groups together with data on the computer programs and lists of coincidences, in return for raw data from their experiments. During the data exchange, errors were discovered in the Maryland programs and in the procedures of other groups. The Maryland errors were acknowledged quickly and carefully corrected. The differences have now been resolved, and independent groups have confirmed the details of the revised Maryland programming. At the request of two research groups, the data exchange was to be private until agreement could be reached on publication. It is regrettable that incorrect and premature reports on the data exchange have appeared in the literature.

Two of the four authors of ref. 1, Steppel and Lee, independently prepared computer programs to process these data.

In summary, results of the magnetic tape computer experiment, the pen-and-ink recorder experiment, and the data exchange indicate that for certain periods there is an excess of coincidences over accidental ones, with significance as great as 5.6 s.d. The use of several independent programs and of the checks in which the searcher did not know what was on the tapes or charts indicates that human observer bias and programming errors have been successfully removed.

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¹ Lee, M. *et al. Phys. Rev. D* **14**(4), 893 (1976).

Radiometric measurement of stratospheric HCl

INTEREST in the effects on atmospheric ozone of man-made chlorine compounds has prompted research into their stratospheric chemistry. As HCl is expected to be the major temporary sink for free chlorine in the stratosphere¹⁻⁴, measurements of its vertical mixing ratio profile could yield useful information as to the major source of chlorine and its future effects on ozone concentrations. We have used a balloon-borne pressure modulator radiometer (PMR) to measure the atmospheric absorption of solar infrared radiation at sunset, and we report here a vertical mixing ratio profile for gaseous HCl for the altitude range 16-39 km.

The major feature of the PMR is a cell containing gas (HCl, in this case) through which the incoming radiation passes⁵. The gas pressure is modulated at about 19 Hz and the mean pressure in the cell chosen to give maximum modulation of cell transmission at HCl spectral line centres. Thus the incoming radiation is selectively modulated in those narrow spectral regions where atmospheric HCl absorbs, and so the pressure modulator cell acts as a filter of very high resolution ($\sim 4 \times 10^{-3} \text{ cm}^{-1}$).

The front optics of the radiometer is a servocontrolled Sun tracking system built by Denver University⁶, which maintains an image of the Sun at the detector. The optical system also contains a rotating chopper which modulates the incoming radiation at 400 Hz and an optical filter which defines a pass band about 300 cm^{-1} wide around 2850 cm^{-1} . This region of the spectrum contains most of the fundamental vibration-rotation band of HCl. The detector, made of lead selenide at ambient temperature, receives radiation which has components at 400 Hz (the wide-band signal), at 19 Hz (the PMR signal) and at $400 \pm 19 \text{ Hz}$ (the side-band signal). The PMR and side-band signals contain the same information but the latter is used for instrumental convenience. The side-band:wide-band ratio is related to the amount of atmospheric HCl in the path between the instrument and the Sun.

The results presented here were obtained on a flight from Holloman USAF base, New Mexico ($32^\circ 52' \text{N}$, $106^\circ 07' \text{W}$) on 17 March 1976. The radiometer remained at its float altitude of about 35.5 km until after sunset and was able to take measurements for solar zenith angles $\geq 90^\circ$. In this way the Sun is viewed through a long atmospheric path, enabling low concentrations of trace constituents to be detected. This technique (limb scanning) also allows vertical resolution in mixing ratio profile to be obtained. Signals for zenith angles $< 90^\circ$ are used to calculate the level of the level of the side-band:wide-band ratio when there is no atmospheric absorption.

The mixing ratio profile has been derived from the data using a computer model which calculates the signals expected from a PMR for a given atmosphere by performing a line by line numerical integration across a Voigt profile for all the HCl spectral lines in the pass band of the filter. The spectral line strengths and Lorentz half-widths used are those given by Toth *et al.*^{7,8}. The results are shown in Fig. 1. Relative errors are indicated; also, at the present stage of analysis, there may be an absolute error of up to $\pm 20\%$ in the

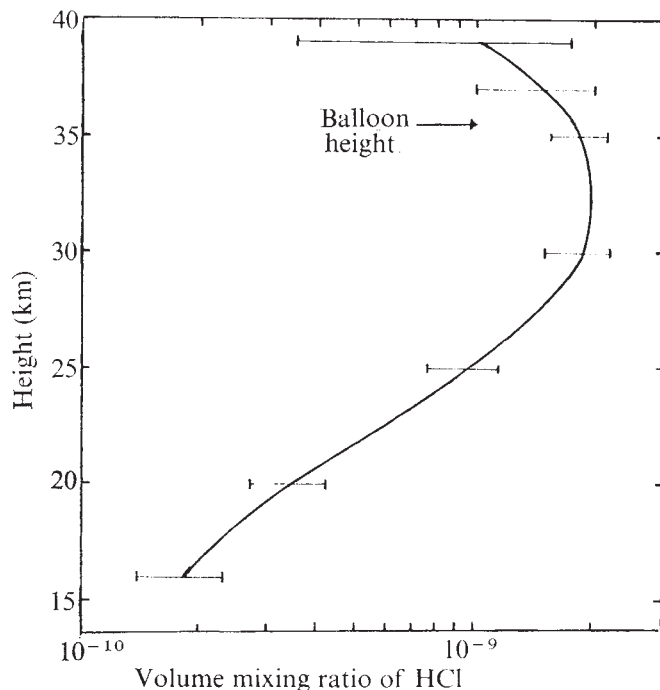


Fig. 1 Vertical profile of the mixing ratio of HCl in the stratosphere calculated from measurements of atmospheric transmission.

mixing ratio. The retrieval takes account of atmospheric temperature structure (measured by radiosondes coincident with the flight) and of the effects of atmospheric refraction. Small corrections have also been made for the estimated effects on the wide-band and side-band signals of other atmospheric constituents which absorb in this spectral region, using the spectral line data tabulated by McClatchey *et al.*⁹

The shape of the mixing ratio profile implies that the major source of HCl in the stratosphere is around 32 km. Other measurements of HCl (refs 10–13) have been based on the

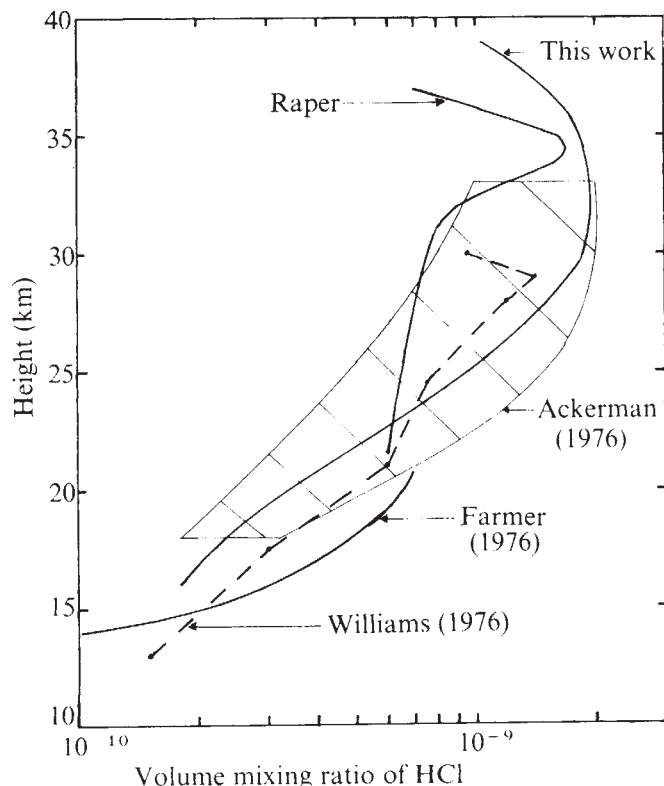


Fig. 2 Mixing ratio profiles of stratospheric HCl. A comparison of the results of this work with other measurements.

measured absorption of individual HCl spectral lines, whereas the PMR uses all the lines in the band. Figure 2 shows that the mixing ratio profile given here is similar to those of Ackerman *et al.*¹¹ and Williams *et al.*¹² but that its shape is different from the most recent profile measured by Raper *et al.*¹³

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Mantle helium in the Red Sea brines

HELIUM isotope studies on terrestrial samples have revealed the existence of two helium components which are clearly distinct from atmospheric helium. The first of these, which we term 'crustal helium', was identified in 1946 in natural gas wells¹. This crustal component is produced by radioactive decay of U and Th to ⁴He, with ³He production by (n, α) reactions on Li; the resulting helium is characterised by ³He/⁴He $\approx 10^{-7}$, one-tenth of the atmospheric ratio². The second component, 'mantle helium', was discovered as 'excess ³He' in deep ocean water, attributed to a flux of primordial helium from the mantle³. Studies of the ³He/⁴He ratio in deep water on the East Pacific Rise⁴ and in helium trapped in submarine basalt glasses^{5,6} have shown that this mantle component is characterised by ³He/⁴He $\approx 10^{-5}$, about 10 times the atmospheric ratio and 100 times the ratio in crustal helium. Basalt glasses from the Western Pacific Lau Basin, the East Pacific Rise, and the Mid-Atlantic Ridge contain trapped helium with similar ³He/⁴He ratios, indicating that mantle helium in at least three areas in which new lithosphere is being formed has a unique and uniform isotopic signature.

The hot brines in the axial rift of the Red Sea⁷ provide an opportunity to sample helium at another plate margin, and to determine the extent to which these brines have interacted with mantle-derived material. Isotopic and chemical studies of the brine water^{8,9} have shown that the source water for these geothermal brines is ocean water of normal salinity which circulates downward through evaporites and horizontally along fissures in the axial basalts to reach the brine-filled depressions. We now report measurements of helium and neon which reveal additional information on the origin of these brines and their relationship to the Red Sea rift.

Our samples were collected on the 1966 Woods Hole CHAIN-61 expedition, using a Nansen-bottle 'piggyback sampler' which recovers samples isolated at *in situ* pressure in copper tubing¹⁰. We analysed two samples taken 2 m apart from the Atlantis II Deep (Station 714, 21°20.5'N, 38°04.5'E); the bottom bottle was 43 m above the bottom in the middle of a 56 °C, 126-m thick layer. From Discovery Deep we analysed three samples collected over a 7-m interval (Station 717, 21°16.7'N, 38°02'E); in this case, the bottom bottle was 42 m

Table 1 Helium, neon and $^3\text{He}/^4\text{He}$ in Red Sea brines (CHAIN 61 Expedition)

	Depth (m)	Concentrations ($10^{-10} \text{ cm}^3 \text{ STP per g H}_2\text{O}$)		He/Ne	$(^3\text{He}/^4\text{He}) \cdot 10^6$	Concentration per atm solubility		
		Helium	Neon			^3He	^4He	Ne
Atlantis II								
714-G7	2121	14.0	0.14	100	12.1	3260	370	1.0
Discovery	2123	(14.8)	—	—	12.2	3470	390	—
714-G8								
717-G10	2153	(14.2)	(0.15)	95	—	—	380	1.0
717-G11	2158	14.1	0.14	105	12.0	3250	370	0.9
717-G12	2160	(13.4)	—	—	12.2	3140	360	—
Red Sea surface water		0.038	0.15	0.25	1.38	1	1	1

Concentrations in brine samples are accurate to 7%, except for values shown in parentheses which are accurate to ~15%. Values for surface seawater (28 °C, 38‰ salinity) are for solubility equilibrium with air at 1 atm (ref. 11). Helium isotope ratios are accurate to 5%, and are reported relative to the ratio $1.40 \cdot 10^{-6}$ for atmospheric helium¹². Sample designations refer to cast and bottle number.

above the bottom in the 44 °C, 164-m thick layer. Conductivity measurements aboard ship showed that the salinity of each sample was the characteristic brine value of 257.5 g kg^{-1} (ref. 9). The samples, each about 20 cm^3 , were sealed in the copper tubing with refrigeration clamps and stored at 4 °C until analysed. They were collected at the same locations as those analysed previously for N_2 , Ar and total CO_2 (ref. 10).

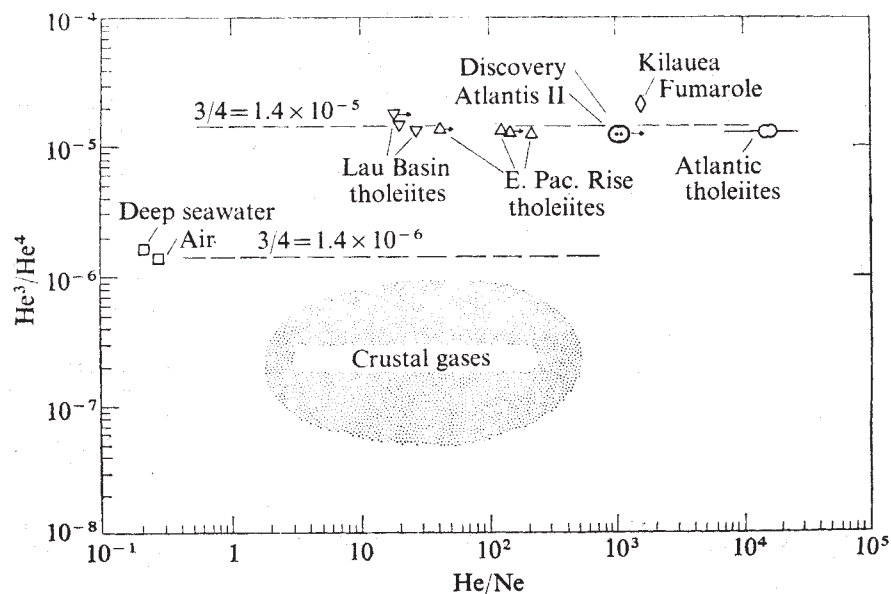
Gases were extracted in a vacuum line made of Corning 1720 glass and stainless steel. In vacuum all samples were clear and colourless, indicating that no contact with oxygen had occurred¹⁰. The extraction and rare-gas purification procedure, which has been tested extensively on seawater samples, collects > 99.9% of the He and Ne. The total system and spectrometer blank (in $\text{cm}^3 \text{ STP}$) is $9 \cdot 10^{-15}$ for ^3He , $1.3 \cdot 10^{-9}$ for ^4He , and $1 \cdot 10^{-10}$ for Ne, < 0.1% of the sample in each case. ^3He , ^4He , and Ne were measured on a double-collecting mass spectrometer⁵ which completely resolves ^3He from HD H_2 . A charcoal U-trap chilled at -195 °C was included in the static volume during analysis to reduce interference from Ar²¹ at mass 20. The $^3\text{He}/^4\text{He}$ ratio was measured in double-collection mode, and the neon was then measured by voltage switching. Air standards were used to calibrate the helium isotope ratios and the absolute amounts of He and Ne as determined by peak height measurements.

The results, summarised in Table 1, show that both brines are highly enriched in helium with a mantle-helium isotopic signature. Because the large variations in salinity in the 15 known brine pools¹³ are due to addition of salt from evaporites and dilution by mixing with seawater⁹, concentrations of gases and their enrichments relative to solubility equilibrium with the

atmosphere are best expressed in terms of gas to water ratios. The measured He concentrations ($\sim 10.5 \cdot 10^{-6} \text{ cm}^3 \text{ STP per g}$ of brine) are thus given as He/ H_2O ratios in Table 1 based on a salinity of 257.5 g kg^{-1} , and are compared with atmospheric-equilibrium solubility ratios in Red Sea surface water at 28 °C, 38‰ salinity, the source-water values estimated from stable isotope ratios⁹ and dissolved Ar in Atlantis II brine¹⁰. On this basis the Atlantis II and Discovery brines are 380 times supersaturated in helium. The ^3He supersaturations are even more spectacular: a factor of 3,300, which can be compared to the highest ocean water enrichment factors of 1.5 observed in deep water adjacent to the East Pacific Rise¹. In contrast to these large helium anomalies, the neon concentrations for both brines agree within errors with equilibrium values, indicating neon enrichments of < 10%.

The $^3\text{He}/^4\text{He}$ ratio in these brines, $1.2 \cdot 10^{-5}$, is similar to that in helium in the glassy rims of recent tholeiitic basalts on Atlantic and Pacific rises^{3,4} (Fig. 1). Since helium in evaporites should be pure 'crustal' helium with a ratio $\approx 10^{-7}$, we conclude that the helium in these brines is primordial helium extracted from basalts in the axial rift during subsurface flow of brine from evaporites to the brine depressions. Comparison with the enrichment factors in brines against original seawater for other elements⁹ shows that ^3He ranks with the trace metals Pb, Mn, Fe and Zn, as the only elements exhibiting enrichments of a factor of > 1,000 in these brines; ^4He is next in order (enrichment ≈ 400), and all other species are enriched by factors < 100. Both the trace metal and major element enrichments are entirely consistent with derivation from evaporites, as shown by comparison of added salts in Red Sea and known

Fig. 1 $^3\text{He}/^4\text{He}$ against He/Ne ratios in Red Sea brines, tholeiitic basalts, Kilauea volcanic gas, and crustal gases. Arrows on points signify lower limits for He/Ne ratios.



salt-deposit brines⁹. In contrast, the similarity of helium isotope ratios in fresh basalt glasses and in the brines indicates that > 80% of the brine helium must have been extracted from basalts. Helium is thus the only component in these brines which has so far been shown to require a derivation from a source other than the Red Sea evaporites.

Comparison with helium concentrations in recent basalt glasses^{5,6} indicates that the He/H₂O ratio in these brines is equivalent to a basalt/H₂O ratio of the order of 2–10 g per g for complete extraction. The slightly lower ³He/⁴He ratios in the brines than in the basalt glasses may be attributed to a 10–20% dilution with radiogenic helium produced in the evaporite deposits by decay of U and Th. Weiss¹⁰ showed that the Ar/H₂O ratio in these brines is close to the original solubility ratio. As the ⁴He/⁴⁰Ar ratio in fresh basalts is about 8 (refs 14, 15), < 1% of the argon in these brines has been derived from basalts.

Figure 1 shows the ³He/⁴He and He/Ne ratios for the two brine samples compared with the values for air, crustal gases¹⁶, tholeiite basalt glasses and a gas sample from Kilauea volcano⁶. For the brines we have estimated the He/Ne ratio in the 'excess' component above atmospheric solubility by assuming that the neon excess is $< 1.4 \times 10^{-8}$ cm³ STP per g H₂O for both brines, that is, 10% of the measured neon concentration. For the injected component in the Atlantis II and Discovery brines, this estimate yields a lower limit for the He/Ne ratio of ~ 1,000; this value, plotted in Fig. 1 shows that Red Sea basalts are more similar in He/Ne ratio to Atlantic than to the Pacific basalts so far analysed.

The relationships in Fig. 1 show that the ³He/⁴He ratio is essentially identical in samples from four plate margins and three oceans in which the He/Ne ratios vary by a factor of 1,000. These large He/Ne variations are due either to an intrinsic heterogeneity in mantle primordial gas concentrations or to a He–Ne separation process which does not fractionate helium isotopes significantly⁶. Helium from the Red Sea brines and other plate margin basalts is also distinct from Kilauea helium, which has a 3/4 ratio ~ 15 times atmospheric, an indication that Kilauea may be tapping a different part of the mantle, more enriched in ³He.

That ³He and ⁴He are the only Red Sea brine components which can be shown unequivocally to be derived from hydrothermal circulation in basalts, rather than in evaporites, has interesting consequences for exploration of possible hydrothermal systems in ocean-ridge basalts¹⁷. The ~ 3,000-fold enrichment of ³He in Red Sea brines relative to seawater shows that ³He (and total helium) should be by far the most sensitive indicators of hydrothermal emanations from ocean-floor basalts. We are now using helium isotope measurements in conjunction with a deeply-towed sample collection system to map hydrothermal plumes in active spreading centres¹⁸.

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The continental crust northeast of Newfoundland and its ancestral relationship to the Charlie Fracture Zone

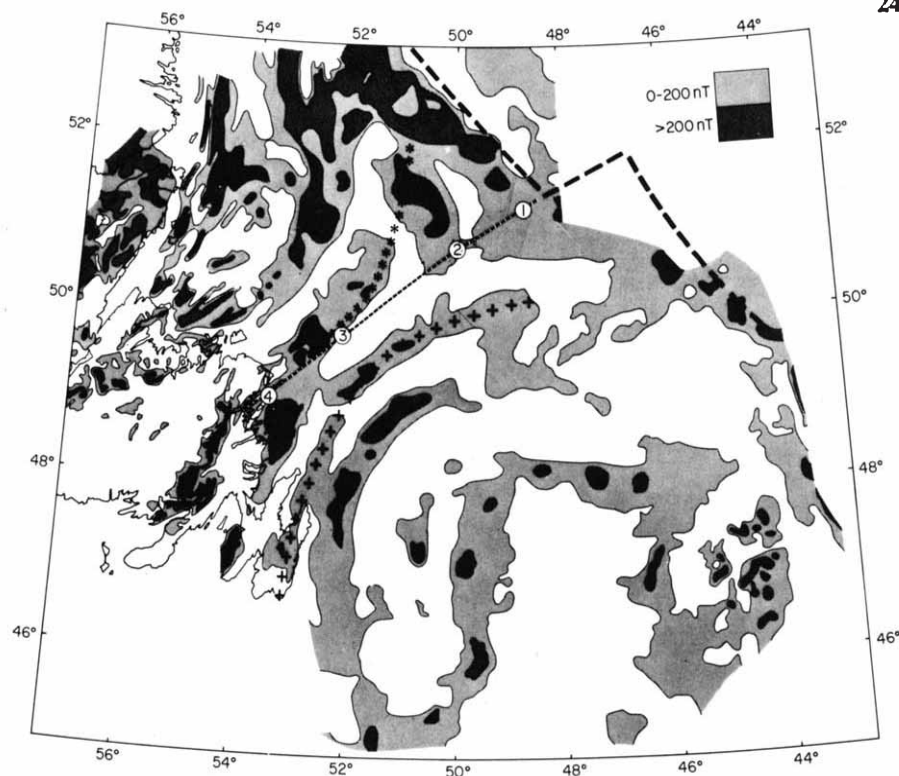
CONTINENTAL edges that are matched in pre-continental drift reconstructions have generally been defined by an isobath. When the isobath chosen is too shallow, isolated bathymetric highs within the 'oceanic' area are interpreted as continental fragments that may consequently be moved at will to fill any gaps in the reconstruction¹. Orphan Knoll (which had a history of uplift, erosion and subsidence² and Flemish Cap³ are two such fragments for which lateral movements across the northeast Newfoundland shelf have been invoked⁴. Detailed bathymetric, magnetic and gravity surveys^{5–6} have confirmed that the entire intermediate depth area of the northeast Newfoundland Shelf inshore of Orphan Knoll and Flemish Cap is continental in nature thereby invalidating reconstructions which assume otherwise. I show here that the offshore extension of the Hermitage–Dover fault in Newfoundland provided the line of weakness along which the Charlie transform fault developed.

Arcuate zones of magnetic anomalies dominate eastern Newfoundland and the northern Grand Banks⁷ (Fig. 1). In the south central Grand Banks, the magnetic highs correlate with basement highs (Fig. 2) from which pre-Devonian metasedimentary rocks⁸ were recovered. Similar rocks on Avalon Peninsula overlie the Precambrian volcanics responsible for the onshore portion of the magnetic highs. These arcuate 'continental' magnetic zones extend into the intermediate depth (1000 m) area of the northeast Newfoundland Shelf (Figs 1 and 2), strongly suggesting that it is underlain by continental crust. In support of this, continental basement can be traced westward along the reflection profile indicated in Fig. 2, from continental Orphan Knoll to the eastern edge of a deep sedimentary basin⁹. A similar seismic reflector can be traced eastwards from land in an entirely continental sequence to the western edge of the same basin⁹. The only possible discontinuity in continental basement from seismic evidence, therefore, underlies the sedimentary basin. But, one of the bands of high magnetic field originating onshore in Precambrian volcanic rocks crosses the basin at the same location as the seismic reflection profile (Figs 1 and 2), thereby bridging the gap. Continuity of continental crust from Newfoundland out to Orphan Knoll is, therefore, corroborated.

The continental area of the northeast Newfoundland Shelf seaward of approximately the 300-m isobath has, however, subsided with respect to the Grand Banks⁷. Although its southern fractured margin is clearly marked by an east–west line of magnetic anomalies at 48°N (Fig. 1), the overall line of dislocation is marked by a gravity high (Fig. 3). This is partially due to the localised sedimentary load on the lithosphere¹¹ but the magnitude of the gravity anomaly can only be explained if the subsided crust has increased in density or has been thinned as a result of margin tectonics (R. A. Folinsbee, personal communication). The inverse correlation between changes in the gravity and magnetic field, which is evident on a local scale over the basin that lies along the line of dislocation, results from the thicker sedimentary section giving an increased load and higher gravity while increasing the depth to basement and, therefore, reducing the magnetic field.

Although the magnetic pattern of Flemish Cap contrasts with that of the Grand Banks, continental basement can be traced between them except for a 40-km wide zone in Flemish Pass³. This distance is, therefore, the maximum possible extent of crustal fracturing or thinning east of the Grand Banks. An overwhelming majority of the area between Orphan Knoll, Flemish Cap and Newfoundland is, therefore, continental, and no major lateral

Fig. 1 The magnetic trends of the continental shelf northeast of Newfoundland based on tracks with maximum spacing of 10 km (refs 5,6). - - -, Line marking 1 offset of edge anomalies, 2 termination of N.W.-trending anomaly pair, 3 divergence between offshore extension of Avalon and Gander (magnetic) trends, and 4 Dover Fault. ****, Localised magnetic trend obliquely crossing N.W.-trending anomaly pair. + + + +, Magnetic trend extending Precambrian volcanics beneath sedimentary basins crossed by Grant⁹ seismic reflection line (see fig. 2). ----, Ocean-continent boundary.



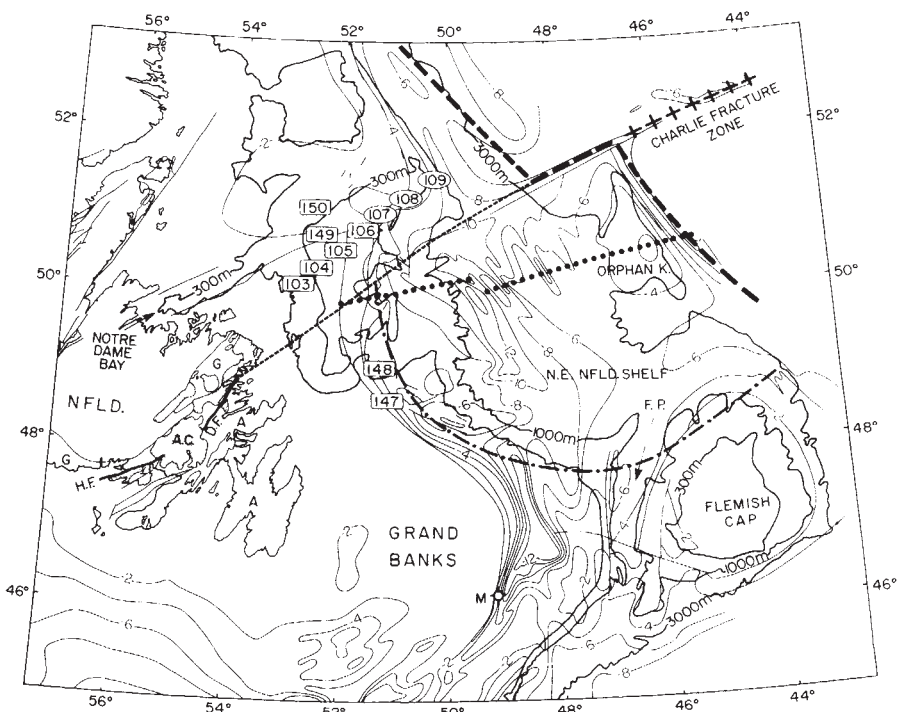
adjustment of the positions of Flemish Cap or Orphan Knoll has occurred. This requires that the continental edge used in palaeogeographic reconstruction should lie east of Orphan Knoll and Flemish Cap as indicated in Figs 1, 2 and 3.

An offset in oceanic magnetic lineations either side of the Charlie Fracture zone¹⁰ correlates well with the northern edge of the subsided northeast Newfoundland shelf and with an offset of approximately 100-km in the gravity and magnetic 'edge anomalies' at the continental margin (point 1 in Fig. 1)^{6,12}. This suggests that the Charlie transform fault can be related directly to an initial offset in the Canadian margin¹⁰. Attempts to identify the seaward extension of one of the faults in Notre-Dame Bay as the ancient line of weakness¹³ controlling that transform fault^{14,15} are only

substantiated as far east as 52°W (refs 9, 16), whereas the older and more fundamental crustal boundary represented in Newfoundland by the Hermitage-Dover Fault can be traced across the shelf to the inshore end of the Charlie Fracture Zone, by a series of disruptions in the geophysical data (points 1-4, Fig. 1).

A pair of positive and negative magnetic anomalies with a northwesterly trend, 100-km long and centred at approximately 51 1/2°N, 50 1/2°W, must overlie continental crust because they are superimposed by subsidiary N-NE trending anomalies that can be traced to pre-Mesozoic features ashore (Fig. 1). 'Basement' velocities of 5.1-5.3 km s⁻¹ obtained over this region have been interpreted as indicating a decrease in the effect of the Acadian orogeny northeast of Newfoundland¹⁷. On the basis of correlation

Fig. 2 Contours on basement (km) (refs 9, 10) and bathymetry of the Newfoundland continental margin. ----, Ocean-continent boundary. - - -, Inshore edge of subsided crust beneath NE Newfoundland Shelf., Grant⁹ seismic reflection line. A.C., Ackley City granite batholith; D.F., Dover Fault; H.F., Hermitage Fault; A., Avalon zone; G., Gander Zone; F.P., Flemish Pass. Numbered rectangles indicate location of refraction lines¹⁷ with high basement velocity; numbered ellipses indicate low basement velocity.



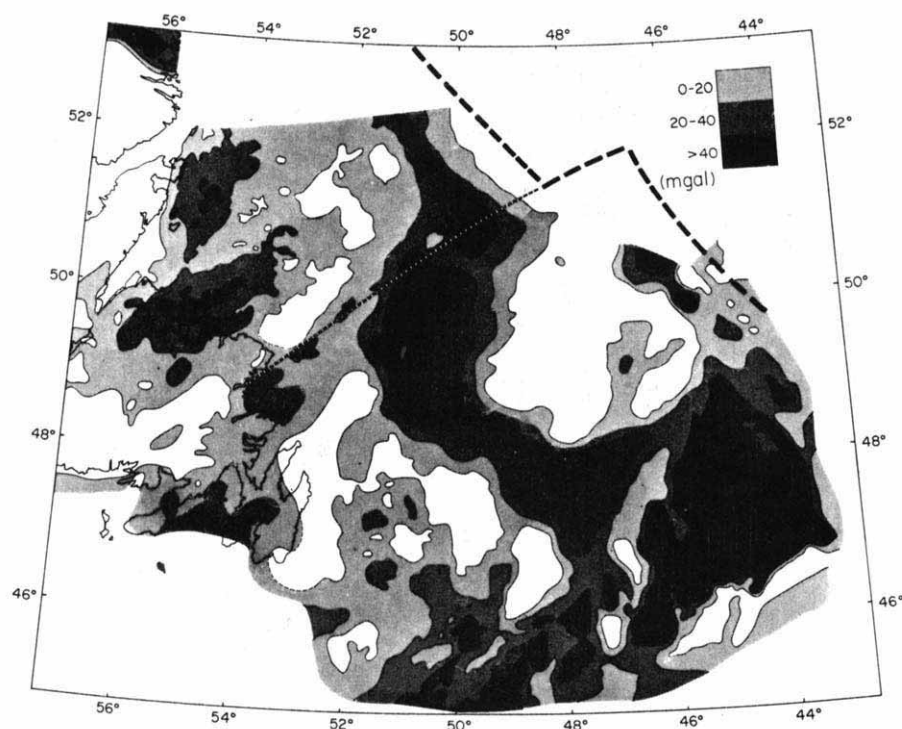


Fig. 3 The gravity trends of the continental shelf northeast of Newfoundland. Bouguer anomalies on land and free air anomalies at sea. Ocean-continent boundary and Dover-Charlie Fault Zone line from Fig. 1.

with magnetic field trends, however, the adjacent higher (Acadian?) velocities ($5.7\text{--}6.3\text{ km s}^{-1}$) probably extend to the continental margin north of the refraction lines. The lower velocity area has in fact subsided along faults subparallel to the continental margin as indicated by the change in depth to seismic basement (Fig. 2) and the increase in wavelength of the transverse magnetic anomalies. The northwest-trending anomalies, which reflect this basement faulting, are abruptly terminated along the southwestward projection of the trend of the Charlie Fracture Zone (2 in Fig. 1).

Further projection of this trend southwestward follows the boundary between the contrasting magnetic anomaly patterns of the Avalon and Gander tectono-stratigraphic zones¹⁸. The arcuate magnetic trends of the Avalon Zone and the Grand Banks veer steadily to the east while the trends of magnetic anomalies emanating from the Central Mobile Belt veer gradually northward (3 in Fig. 1).

The calculated depths to an intermediate crustal layer of velocity $6.9\text{--}7.6\text{ km s}^{-1}$ (ref. 17) can accommodate a 2-km normal fault¹⁴ between refraction lines 148 and 149 (Fig. 2). The reduction in amplitude and increase in wavelength of magnetic anomalies that would be associated with such a fault, are found at the eastern edge

of the high magnetic zone that enters Newfoundland on the western side of the Dover Fault (3 and 4 in Fig. 1).

The Dover¹⁹ and Hermitage Faults²⁰ define the boundary between the Gander and Avalon Zones (Fig. 2). The fundamental nature of this boundary established on land, combined with the ability to trace its extension, by means of geophysical boundaries (1-4, Fig. 1), to an offset in the continental margin and the western end of the Charlie Fracture Zone, indicates that the Hermitage-Dover Fault Zone is ancestral to the Charlie Fracture Zone.

Cherkis^{15,21} has suggested from magnetic field data that the line of weakness along which the Charlie transform fault developed is also the location of the Hercynian (Variscan) Front. Although magnetic trends in Europe have been interpreted as associated with Hercynian deformation²², the seaward extension of any trend (magnetic or gravity) that might correlate with the Hercynian Front itself, is not apparent west of Ireland^{23,24}. On most palaeogeographic reconstructions of the North Atlantic, however, those magnetic trends in Europe attributed to Hercynian deformation are roughly concentric with the arcuate magnetic zones of the Grand Banks and the Avalon Zone (Fig. 4). If the Hercynian Front lies at the northern limit of those magnetic zones, as in Europe, the implication would be that the Hercynian Front follows the Hermitage-Dover Fault Zone, but this suggestion can be opposed on geological grounds. Work in progress on clearer identification of those potential field trends associated with strictly Hercynian features in North America and Europe indicates that the arcuate zones in Newfoundland and Iberia are Precambrian rather than Palaeozoic. Hercynian trends would, therefore, be associated with disruption of the magnetic pattern rather than the pattern itself.

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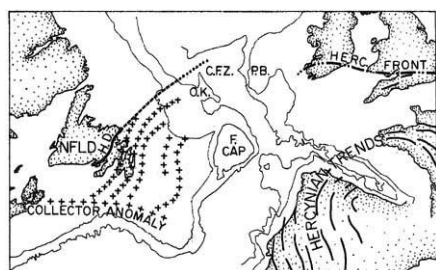


Fig. 4 Trans-Atlantic correlation between north-east Newfoundland and Hercynian trends. The Collector Anomaly marks the southern limit of the Avalon arcuate magnetic zones. Juxtaposition of N. America and Europe is based on a morphological fit between the two margins in which no movement of continental fragments has been closed²⁵. No other justification is claimed for this reconstruction, and the roughly concentric nature of trends prevails if other reconstructions are used. C.F.Z., Charlie Fracture Zone; O.K., Orphan Knoll; F. Cap, Flemish Cap; P.B., Porcupine Bank.

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Preliminary palaeomagnetic results from Makapansgat and Swartkrans

THE South African hominid sites have proved difficult to date since radiometric and fission track methods have yielded no results, and faunal and geomorphological methods provide only wide limits. We describe here preliminary palaeomagnetic results which suggest that polarity stratigraphy can be used at these sites, and which indicate an age of between 2.8 and 3.3 Myr for Member 2 of the Makapansgat Formation.

Samples were collected at the Swartkrans and Makapansgat Limeworks sites. Both sites were described by Brain¹ who has subsequently revised his interpretation of Swartkrans² and in conjunction with Butzer has suggested new nomenclature^{2,3}. Detailed mapping at Makapansgat by one of us (T.C.P.) has led to a revision of the stratigraphy which will be briefly described here. Full details will be published elsewhere.

The Makapansgat Formation constitutes the cave filling at the Makapansgat Limeworks hominid site, the cave being a solution cavity in the Malmani Dolomite of the Transvaal Supergroup. The lowest member (Member 1) is a contaminated travertine, locally bone-bearing, deposited on an irregular dolomite floor and locally tilted and distorted. Member 2, which forms the lower part of Brain's Lower Phase 1 unit¹, is a bone-bearing brownish-red well-bedded deposit of silt to fine sand grade material coarsening upwards, and is 2-10 m thick. It was deposited subaqueously within the cave and there seem to have been two depositories, one to the east and one to the west, separated by a floor high but controlled by a common water table level close to 1458-m elevation. Member 3, formerly the Grey Breccia of Lower Phase 1 is a thin (0.5-2.0 m) deposit consisting largely of a densely packed accumulation of bone cemented by calcite. Much of the Makapansgat fauna is derived from this Member which unconformably overlies Member 2. Member 4, formerly the Pink Breccia of Upper Phase 1, is a thick (3-15 m) deposit of pinkish silty sand material containing many angular chert and dolomite fragments, the larger of which are probably derived from roof falls, and local concentrations of bone. Member 5 (formerly the Red, or Phase II, Breccia) constitutes a locally well-stratified brownish-red silty sand matrix containing abundant chert and dolomite fragments and resting unconformably upon an eroded surface of Member 4. This unconformity probably represents a considerable lapse of time. Bone, often poorly preserved, is scattered throughout Member 5. Most of the remains of *Australopithecus africanus* were recovered from Member 3, but the posterior part of one cranium was recovered from Member 4, and one equivocal maxillary fragment from Member 5.

Forty palaeomagnetic samples were collected. The very varied physical nature of the rocks dictated a variety of collecting techniques. At Swartkrans 15 block samples, oriented magnetically, were collected. At Makapansgat 18 samples were cored and four (all from Member 1) were collected as blocks; all of these were oriented with a theodolite using Sun sights. Three samples from soft red muds (Member 2) in the deepest part of the cave were encased in small plastic cubes and oriented magnetically. Member 3 was not sampled, due to the poor mechanical properties of the rock at the accessible localities. All measurements were made in the Salisbury laboratory using a spinner magnetometer described by van Oorschot and Ridler⁴. Routine AF cleaning was used and a stable component was isolated at typical peak fields of about 20 mT; many samples showed evidence of rotational remanent magnetisation⁵ at higher fields. Stable directions were arbitrarily classified as normal (N) or reversed (R) if they were within 40° of the relevant dipole field direction and as intermediate (I) if they fell outside this limit. Samples which showed erratic demagnetisation, or which exhibited internal disagreement between specimens were classified as giving no result (X).

The results for Swartkrans are shown in Table 1. One sample broke up during preparation and only 14 are shown in the table. Of these, eight are unstable or inconsistent, four are intermediate and two are normal. The large number of unstable samples is disappointing and the proportion of intermediate samples is higher than would be expected for a geomagnetic field effect. Even if the two normal samples represent an original primary magnetisation we do not feel that any chronological conclusions should be drawn from these data.

Table 1 Polarity data for Swartkrans

Unit	Sample No. (PM)	Polarity of samples
Member 1, Unit B1 Pink Breccia	1, 2	X, I
Member 1, Unit B2 Pink Breccia	3, 4	I, I
Member 1, Unit C Orange Breccia	5, 6, 7	X, X, X
Member 2, Inner Cave Stratified Brown Breccia	8, 9, 10, 11	I, X, X, X
Member 2, Outer Cave	13, 14, 15	N, X, N

Samples from Makapansgat showed much greater stability: the results are given in Table 2 and shown schematically in Fig. 1. Member 1 is entirely reversed. The beds at the point of sampling are tilted and when the palaeomagnetic directions are corrected for the tilting they become significantly more scattered (although still reversed), suggesting that the magnetisation was acquired after tilting. Member 2 is horizontally bedded and lies with minimum distortion upon the tilted Member 1, indicating that the deposition of Member 2 also occurred after the tilting. In Member 2 the lowest sample in the western depository is intermediate, whilst the remaining 5 horizons (containing 12 samples) are all normal. Low in Member 4 an intermediate horizon occurs, while high in Member 5 two reversed horizons occur followed closely upwards by two intermediate horizons.

The simplest interpretation of these results is that there is an upwards reversed to normal transition low in Member 2 which is otherwise entirely normal. An intermediate horizon low in Member 4 suggests a transition back to normal, but since Member 3 was not sampled it is not clear whether this is the transition immediately following that in

Table 2 Polarity data for Makapansgat

Unit	Sample No. (MT) by horizons	Polarity of horizon
Member 5	25	I
	24	I
	23	R
	18, 19	R
Member 4	20, 21, 22	I
Member 2, east	15, 16, 17	N
	12, 13, 14	N
Member 2, west	8, 9	N
	5, 6, 7	N
	10	N
	11	I
Member 1	2	R
	4	R
	3	R
	1	R

Member 2. A reversed section high in Member 5 followed by two intermediate samples suggests another reversed to normal transition. The placement of these polarity changes in the polarity time scale must depend upon other evidence for the approximate age of the beds.

Two other lines of evidence are available. The first is geomorphological. Partridge, using estimates of nickpoint migration and valley widening, calculates an approximate age for the cave opening at Makapansgat of 3.7 Myr (ref. 6). This is the time from which cave filling became possible, and is therefore a maximum age for the deposits. The second is faunal. Cooke⁷ has used suid evidence to suggest that the Makapansgat fauna is "in the vicinity of 2.5 to 3.0 million years old" (quoted by Tobias⁸ in a review of the evidence). White and Harris⁹ have recently suggested

that "the Makapansgat (suid) fauna is equivalent in age to Members B-C of the Shungura sequence" in Ethiopia. The palaeomagnetic stratigraphy of Shuey *et al.*¹⁰ for the Shungura Formation places the base of Member B, the base of Member C and the top of Member C at about 2.9, 2.6 and 2.4 Myr, respectively. Vrba has used bovids to suggest tentatively that the Sterkfontein type site is "somewhere between 2 and 3 Myr" in age (personal communication) whilst Wells¹¹ feels that the Makapansgat fauna slightly predates that of Sterkfontein. The published faunal evidence is still rather scanty, but seems consistent with an age of between 2.5 and 3.0 Myr for Member 3, from which the bulk of the fauna derives. These figures therefore set an approximate younger limit for the age of Member 2 in which the polarity change occurs.

With these limits, the upwards transition from reversed to normal polarity low in Member 2 must have an age of 3.32, 2.94 or 2.80 Myr if we use Cox's¹² polarity time scale. The next closest Transitions in the same sense are at 3.90 Myr (Cochiti) and 2.13, 1.98 or 1.79 Myr (Réunion or Olduvai) using the same time scale, and are well outside the limits set by the geomorphological and faunal evidence. It must be emphasised that the palaeomagnetic argument depends heavily upon the limits provided by the other evidence, but if these limits are valid it becomes possible to state that Member 2 of the Makapansgat Formation lies within the Gauss normal epoch and has a maximum age of between 2.8 and 3.3 Myr. The assumed transition low in Member 4 could then be one of those higher up within the Gauss or at its upper boundary. The reversed and intermediate directions high in Member 5 occur after what is believed to be a considerable lapse of time and may be associated either with the Réunion or the Olduvai events.

All of these conclusions depend upon the cleaned palaeomagnetic directions being primary. Full details will be published elsewhere, but the demagnetisation behaviour both in direction and intensity suggests that a primary component has been isolated. Moreover, the mean directions of the normal samples in Member 2 both at sample and at horizon level lie closer to the dipole field than to the present-day field at Makapansgat, which suggests that recent remagnetisation has not occurred.

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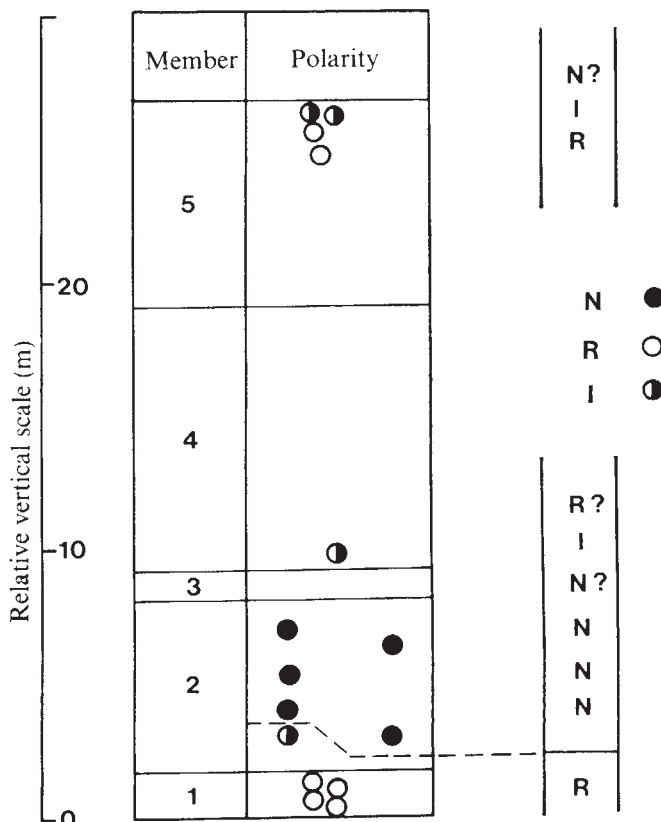
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New aspects of Middle Palaeolithic variability in western Europe

OBSERVATIONS are presented here on quantified aspects of Middle Palaeolithic variability which have not been studied systematically so far. The findings relate to differing degrees of secondary modification of lithic artefacts between assemblages. The variations correspond with distinct toolmaking entities defined for early Würm horizons in western Europe. They offer new perspectives, however, on the interpretation of Middle Palaeolithic inter-assemblage variability by suggesting the possible importance of non-cultural causes for some of this variability.

Distinctive Middle Palaeolithic assemblages are found within stratified sequences or between individual sites. These differences have been expressed quantitatively by comparing lithic assemblages through the classificatory method developed by Bordes *et al.*, particularly the polymodal frequency distribution of the racloir index, to which correspond characteristic tool type clusters¹. Two main industry groups have been defined: le Moustier, which consists of the Denticulate, the Acheulian Tradition (or MTA) types A and B and the Typical Mousterian; and the Charentian, including the Ferrassie and Quina. This classificatory arrangement is the most widely accepted at present.

Controversy persists, however, regarding the significance of 'polytypic' Middle Palaeolithic phenomena. Resolution is theoretically important because of the increased knowledge which may be obtained on the behaviour of Neanderthal populations. Bordes interprets the different industry types as the manifestation of separate toolmaking traditions manufactured by distinct communities often coexisting within a same area². Mellars supports the conclusion that most of the traditions are patterned chronologically³, while Binford favours a functional interpretation: the different tool type clusters are specialised toolkits for the performance of various tasks by culturally similar populations⁴.

The evidence discussed here is derived from some 120 assemblages excavated and sorted typologically by well-known workers over the last 25 yr. The method used concentrates on variable features of broad artefact groups and relies on the Bordes classificatory system. The basic observation unit includes all regularly retouched tools (or implements)—all racloirs types, denticulates and notches, bifaces and types nos 6–8, 30–35, 40–41 and 53–60 of the Bordes type list—whose frequencies are calculated against the number of implements plus all irregularly retouched, utilised or blank Levallois and non-Levallois flakes and blades. A distinction is therefore made between regularly transformed and utilised tools and flakes casually modified or utilised or without macroscopic wear traces. Sub-categories are defined, including (1) 'blanks and utilised flakes', nos 1–5 and 45–50, of the list; (2) non-Levallois unretouched flakes; (3) 'Levallois implements', or the number of pieces among Levallois flakes which are modified into implements; (4) racloirs and (5) denticulates and notches. Ratios of flake tools and blanks to cores per assemblages are also included.

Figure 1 shows the mean and range of implement frequencies for each Middle Palaeolithic industry type. The observed separation into two clusters agrees with a sorting of assemblages into le Moustier and Charentian groups. A maximum distance is found between the Denticulate and Charentian industries and the plotted ranges are discontinuous. The Typical and MTA show intermediary values overlapping with other industry types. A similar pattern was noted among individual sites sequences, for example, at Combe-Grenal, Hauteroche, Caminade, Armand Chadourne and le Maras, suggesting the presence of a recurrent, consistent trend.

Table 1a compares averages of frequency variations for artefact sub-categories among Middle Palaeolithic industries. The differential patterns are consistent with those shown for implement frequencies. Values for Levallois implements, though

generally higher, conform with previously observed tendencies. In Table 1b, ratios of flakes and blanks to cores again conform with the earlier findings, the highest values being associated with the Charentian.

The data suggest that differential blank production and secondary modification are correlated. Higher blanks and flake implements to core ratios may be due to more exhaustive flaking of flint nodules, although other factors may be involved. Taken together, both could be attributed to non-cultural causes, for example, the influence of environmental circumstances inducing more economical uses of lithic material, as illustrated by the following studies.

In one case, it was shown that scarcity of flint in some crystalline regions determined increased secondary flaking and the development of primary flaking techniques making optimal use of available flints⁵. Another study has contrasted within the same le Moustier traditions, the open-air summer camps of short duration with the more sedentary rockshelters or caves settlements. In the latter, more Levallois blank pieces were secondarily transformed into implements than in the former.⁶

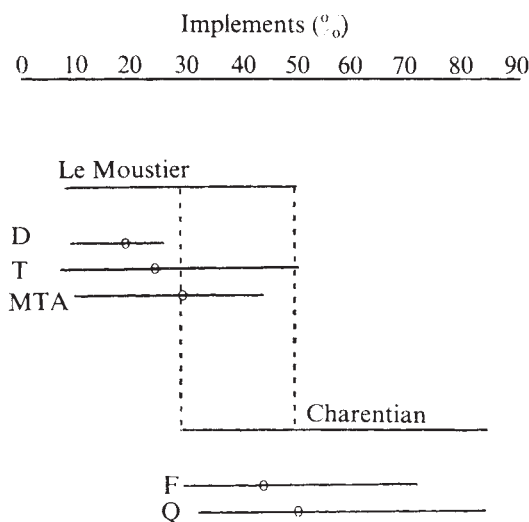


Fig. 1 Average implement frequencies and ranges for Middle Palaeolithic industries. D, Denticulate; F, Ferrassie; MTA, Moustérien de Tradition Acheuléenne; Q, Quina; T, Typical; I, implement frequencies. Data are derived from artefact counts of sites in western Europe: Grainfollet (shelter, beach), Méru, Oissel, Houpeville (séries rousse et claire), Bihorel (III), Hamel (8), Busigny, Laigny (C), Evreux (6), Gros-Monts (2), Goderville (s. lustrée) Tillet (s. café-au-lait), Marcoing, Oldbury, Combe-Grenal (1, 6–38, 40–43, 46, 50–52, 54), Pech de l'Aze, locus I (3, 4F, A, B, C, 5, 6, 7), locus II (3, 4B, 4F, 4C2), Hauteroche (1, 3), Bouheben (1, 1'), Nantet I, Abri Chadourne (A, B, C, D), Caminade (1, 2, 3, 3s), la Rochette (7), Las Pénélous, Mas-Viel, Vauzelle, Plateau Baillard (1, 2), Chinchon, le Dau, l'Ermitage, Petit-Puymoyen (Grotte, 2, brèche, 3), Baume-Vallée, le Madriat, St. Maurice-sur-Loire, le Maras (1, 3, 4, 5), Ranc-Pointu, le Figuier, Blanzay, l'Hortus (16–21A, 18–25, 7–17), Rigabe (G), Bas-Guillotte, Grotte Tournal (1, 2, 4, 5), Abri Romani (4–8, 9–9a, 10–13), San Francesco, Kokkinopilos (Greece).

These explanations, though probably correct in their context, do not apply here for various reasons. First, most of the sites represented by the data are from regions where flint or equivalent raw materials are regularly available. Second, the most pronounced quantitative differences involve industries such as the Denticulate and the Charentian which occur mostly in rockshelter or cave sites in Western Europe, often within the same stratified successions. And third, more significantly, average differences in primary and secondary flaking co-vary with defined Middle Palaeolithic traditions, instead of forming non-cultural facies within them. These differences apply to Levallois and non-Levallois flakes. Consequently, the variations do not seem to be related to environmental circumstances such as a settlement dichotomy or regional scarcity of flint.

Table 1 Comparisons of *a*, major artefact class averages and *b*, ratios of flakes and flake tools to cores for each Middle Palaeolithic industry

Middle Palaeolithic industries	Implements	<i>a</i> Utilised and blanks	Levallois implements	Non-Levallois plain flakes	<i>b</i> Flakes and flake tools ratios
D	17.1	24.7	35.0	57.7	20.2
T	22.7	26.5	36.4	50.8	22.5
MTA	25.6	25.4	49.4	48.5	14.7
F	43.0	21.4	49.6	35.4	31.0
Q	49.8	10.6	58.6	39.5	33.3
Sample averages	29.9	22.0	43.3	47.9	—
N assemblages	120	120	98	120	94

On the other hand, it would be difficult to test independently a hypothesis stating that these new observations constitute an additional set of cultural norms from distinct toolmaking traditions. Other quantitative data give little support for a 'cultural' hypothesis.

First, the implements frequency histogram for the entire sample is continuous and unimodal, suggesting a single phenomenon, whereas a polymodal model, analogous to that for the racloirs index, would be expected for distinct toolmaking norms. Second, the scatter diagram (Fig. 2) shows that high implement frequencies depend mostly on increased racloirs frequencies. Racloirs along with denticulates and notches are the most frequent Middle Palaeolithic implement classes. Their relative variations provide diagnostic criteria for the identification of most early Würm traditions in the Bordes system. Racloirs predominate strongly in the Charentian, denticulates and notches in the Denticulate and type B Acheulian Tradition Mousterian. We show above that the variations in implement frequencies revealed by this study are due to increases in racloirs manufacture rather than in miscellaneous implement forms. If preference for racloirs expresses the design norms of Charentian populations, it should be emphasised that the manufacture of stylistically alternative forms is never commensurate with that of racloirs in other toolmaking traditions. This analysis is the first to measure this non-equivalence or quantitative asymmetry. It confirms but amplifies the conclusion that racloirs constitute the most dynamic artefactual element in Middle Palaeolithic assemblages and also suggests that more than cultural preferences may be involved in high racloirs frequencies.

The discovery of new dimensions of variability adds to the number of open problems identified with the Middle Palaeo-

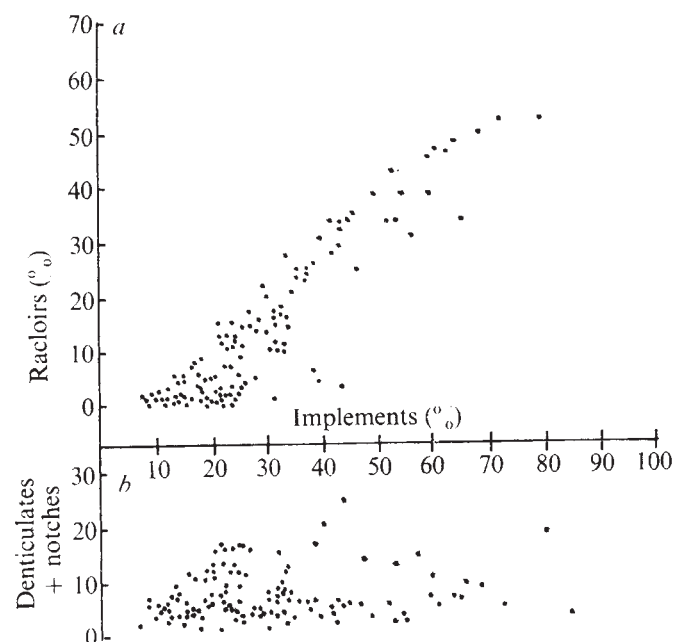


Fig. 2 Scatter diagram of implement frequencies (I) with *a* racloirs (R) frequencies and *b* denticulates and notches (D : N) frequencies. Only *a* shows positive association.

lithic. These problems are present at a descriptive (statistical) and interpretative (anthropological) level: (1) to what extent do the graphic patterns for lithic assemblages represent either one or more original populations? (2) The issue of whether inter-assemblage differences can be interpreted in terms of the existence of distinct identity-conscious groups or of several discrete activities within a single one remains unresolved. Both interpretations are theoretically reasonable but neither has been subjected to sufficiently stringent tests.

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'Modern' lizard from the Upper Triassic of China

LIZARDS are the most numerous, varied and widespread of living reptiles. An essentially modern lizard fauna has existed throughout the Tertiary and members of many modern lizard families are known from the late Cretaceous. Earlier in the Mesozoic, very few fossils of lizards are known. Lizards from the Upper Jurassic may be related to the ancestors of modern groups, but are placed in distinct families. Lizards previously described from the Upper Triassic of Europe¹ and North America² are considerably more primitive, retaining many cranial features in common with their ancestors from the Upper Permian and Lower Triassic³. We give here the first description of an Upper Triassic lizard with an essentially modern cranial morphology. This specimen is interesting with respect to the origin, differentiation and zoogeography of the modern lizard groups.

Class Reptilia
Order Squamata
Suborder Lacertilia
Infraorder Incertae Sedis
Family Fulengidae n.

Fulengia youngi, gen. nov. et. sp. nov.

Holotype: CUP 2037, skull, lower jaws and associated vertebra; collection of the Catholic University of Peking, now housed in the Field Museum of Natural History, Chicago. Collected by Fr E. Oehler, SVD in 1948 or 1949; part of a rich fauna of therapsids and archosaurs⁴.

Locality and horizon: Ta Ti, Lufeng Basin, near K'un-ming, Yunnan Province, China. Dark Red Beds, Lower Lufeng series, Upper Triassic, equivalent to the Norian or Rhaetic of Europe⁴. The generic name is an anagram based on the locality from which the specimen

was collected; the specific name honours the work in this region by Dr C. C. Young.

Diagnosis: a combination of the following characteristics distinguishes this form from other lizards previously described from the Mesozoic and Cainozoic: median

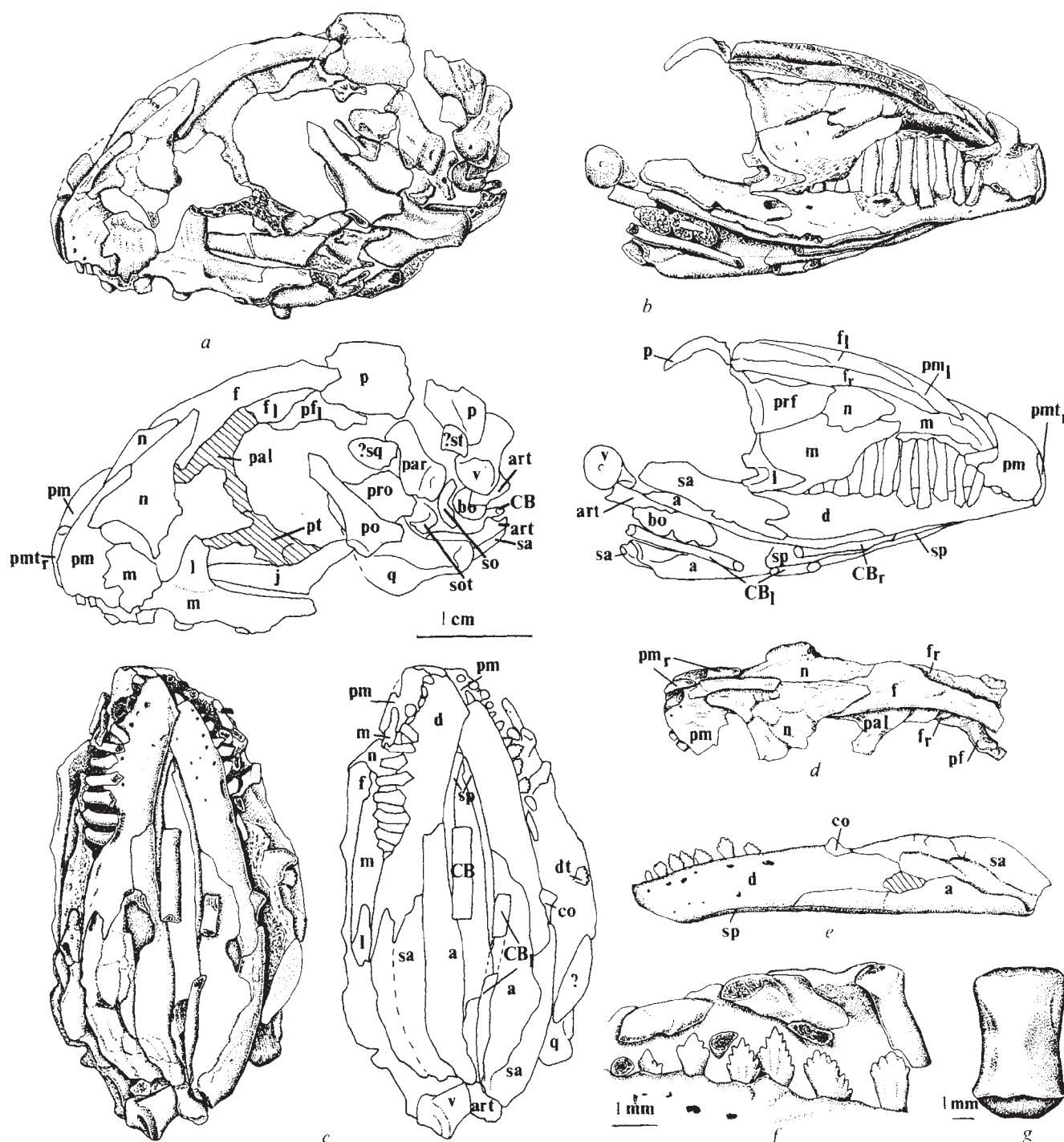


Fig. 1 *Fulengia youngi* gen. nov. et sp. nov., holotype, CUP 2037. *a*, Left side of skull; in outline drawing areas only questionably identifiable have been omitted, diagonally shaded area distinguishes palatal elements crushed into plane of skull roof; dotted line indicates approximate position of division between lacrimal and maxilla; dashed line indicates anterior margin of quadrate. *b*, Right side of skull; most of the right maxilla has been displaced posteriorly relative to the maxillary teeth. *c*, Ventral view of skull with lower jaws covering palate; broken line on surangular indicates normal dorsal extent of displaced angular. *d*, Dorsal view of anterior portion of skull. *e*, Lateral view of left lower jaw. *f*, Teeth of left dentary (below) and maxilla; teeth seen in section are from the more anterior portion of the maxilla that has been telescoped between the other elements. *g*, Associated vertebra in ventral view presumed anterior end uppermost. (*a-e*, $\times 2$; *f*, $\times 8$; *g*, $\times 4$). *a*, Angular; *art*, articular; *bo*, basioccipital; *CB*, first ceratobranchial; *co*, coronoid; *d*, dentary; *dt*, displaced tooth; *f*, frontal; *j*, jugal; *l*, lacrimal; *m*, maxilla; *n*, nasal; *p*, parietal; *pal*, palatine; *par*, paroccipital process; *pf*, postfrontal; *pm*, premaxilla; *pmt*, premaxillary tooth; *po*, postorbital; *pro*, prootic; *pt*, pterygoid; *q*, quadrate; *sa*, surangular; *so*, supraoccipital; *sot*, sphenoccipital tubercle; *sp*, splenial; *sq*, squamosal; *st*, supratemporal; *v*, vertebra; subscripts *l* and *r* refer to bones from left and right side where there is a possibility of confusion. In the case of the frontal, they indicate broken elements of a median bone.

frontal; paired premaxillae with long nasal processes; large upper temporal opening with a margin partially formed by the postfrontal; postorbital triradiate and nearly vertical; lateral articular surface of paroccipital process large, indicating support of quadrate and squamosal; lacrimal reduced; approximately five teeth in the premaxilla, nine in the maxilla and 13 in the dentary; teeth fully pleurodont, closely resembling those of the modern genus *Iguana iguana*; laterally compressed, cusped crowns whose anterior margin is angled toward the midline; low coronoid process, approximately midway between articular and symphysis; splenial extending from behind coronoid to symphysis; vertebrae weakly procœlous; skull of only known specimen 38 mm long. The absence of comparative material from the early Mesozoic makes composition of a proper differential diagnosis impossible. There is no clear basis for determining which characters may be diagnostic at the level of species, genus or family, although it is probable that dental characteristics and relatively large size are typical of the species or genus, but not family. In spite of the probable exclusion of this genus from previously recognised lizard infraorders, we do not consider it desirable to create a new infraorder on the basis of a single incomplete skull.

The skull (Fig. 1) is flattened laterally and telescoped from back to front, as well as having some individual elements further displaced. Simmons⁴ indicates that many specimens from this locality give the impression of being coprolitic; passage through the digestive system may account for the damage to this specimen. Although the bones identified in Fig. 1 are for the most part clearly defined, in other areas of the specimen it is not possible to differentiate bone from matrix. Some of the bones are broken and deeply fissured, but there is little difficulty in differentiating sutures from breaks.

The configuration of most of the bones of the roof of the skull can be seen from one side or the other. Important exceptions are the parietal and squamosal. The area of the parietal is too poorly preserved to show whether or not the bone is paired, but it appears to be fused medially. The parietal foramen appears to have been located at the juncture between the frontal and parietal. The position of the paroccipital process defines the extent and orientation of the squamosal, but the specific relationship with the quadrate (the dorsal portion of which is covered by the proötic and postorbital) cannot be determined.

With the exception of brief reference to more advanced forms¹, our knowledge of Late Triassic lizards is limited to the specialised gliding forms from Great Britain and North America in the family Kuehneosauridae. In spite of their extreme postcranial specialisation, these genera retain a skull morphology similar to that of the late Permian and early Triassic Paliguanidae³ from South Africa. The oldest described members of the infraorders Gekkota, Iguania, Scincomorpha and Anguimorpha are from the late Jurassic of Europe and China⁵. The only living infraorder of lizards not recognised in the Upper Jurassic is the Amphisbaenia, known only since the early Tertiary⁶. Differentiation of the modern infraorders presumably began early in the Jurassic or even in the Upper Triassic.

Fulengia does not show the particular combination of features by which members of the Gekkota, Anguimorpha or Amphisbaenia are recognised. What is known of the skull, as restored (Fig. 2), resembles in a general way the pattern of primitive members of the Iguanidae, some large teiids and members of the extinct scincomorph families recently described from the Upper Cretaceous of North America and Mongolia⁷. Most of the features that it shares with these groups, however, are clearly primitive; for example, the presence of a large dorsal temporal opening, a complete postorbital bar and a vertically oriented, tri-radiate postorbital, and do not support assignment to any of these families. The massive lower jaw and the tiny

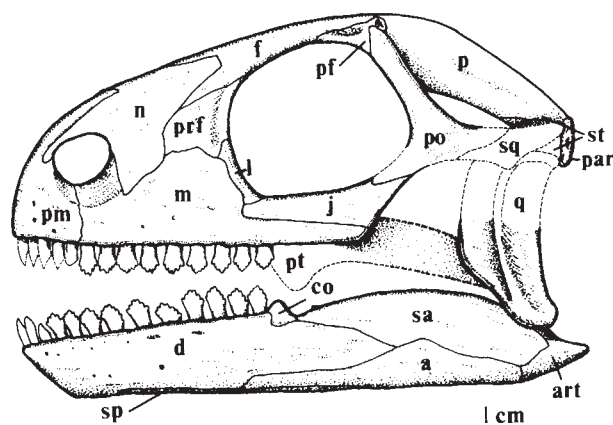


Fig. 2 *Fulengia youngi*, gen. nov. et sp. nov., reconstruction of the skull in lateral view $\times 2$. Bones and teeth shown in broken lines are either missing or covered by other elements in the specimen. They are reconstructed on the pattern of the living *Iguana*. Abbreviations as in Fig. 1.

coronoid distinguish this genus from members of all living reptile infraorders.

The dentition of *Fulengia* (Fig. 1f) is presumably a specialised characteristic and probably less important than the pattern of the skull bones in establishing infraordinal relationships. Multicusped, laterally compressed tooth crowns similar to those of *Fulengia* are known in numerous living lizards belonging to diverse taxonomic groups. Where stomach contents have been studied⁸, such a pattern is associated with a primarily herbivorous diet. Accepting a skull:trunk ratio similar to that of living iguanids with similar skull size⁹ and length:weight ratios determined by Pough¹⁰, the weight of this specimen would be approximately 80 g—towards the top of the range for primitive insectivores, but near the bottom of the range for herbivores¹⁰. Dental specialisation toward a herbivorous diet and relatively large size are features not expected in primitive lizards¹⁰ and particularly not in the group(s) ancestral to the advanced infraorders, most of which are represented by small, apparently insectivorous, forms in the late Jurassic. The relatively large size of the skull was probably an important factor in its preservation, in view of the extremely poor fossil record of other, smaller, lizards in the early Mesozoic.

Fulengia itself can be viewed as a precociously specialised genus, divergent from the main line of lizard evolution. Other members of the family to which it belonged may have been closer to the ancestry of modern groups. The Fulengidae may (1) be related to the stock from which the known iguanians arose; (2) represent an earlier stage, close to the ancestry of most lizard infraorders, or (3) exemplify an independent achievement of some modern lizard features from the primitive eolacertilian stock. The absence of comparable forms in the Triassic and early Jurassic makes it difficult to establish the relationship of *Fulengia* more specifically.

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Adaptive reduction in permeability of avian eggshells to water vapour at high altitudes

WE report here the existence of an altitudinal cline in permeability of eggshells to water vapour for eggs of a North American species of bird. This pattern of geographic variation in permeability of eggshells seems to have arisen as an adaptive response to altitudinal variation in the diffusivity of water vapour through air. Diffusivity of atmospheric gases has not previously been widely recognised as an important component of the physical environment to which organisms are exposed, and our findings provide further evidence for the pervasiveness of natural selection.

A bird egg loses water continuously during normal incubation owing to the outward diffusion of water vapour through pores in the calcareous shell^{1–3}. The loss of a certain amount of water from the egg is essential for the normal development and hatching of the embryo⁴, for the escape of water allows an air space to form in the blunt end of the egg⁵ and this space is the source of air for the first breaths taken by the embryo before hatching⁴. Reserves of water within the egg to support the metabolism of the developing embryo are fixed in amount, however, and the loss of large quantities of water from the egg could be detrimental⁶. Thus, porosity of the eggshell is delicately adjusted (in an evolutionary sense) to assure that only as much water is lost from the egg during incubation as is required for formation of an air space of the necessary volume^{3,7}.

Eggs laid by birds breeding at high altitudes face a potential disruption of the balance between water loss and water conservation³, simply because molecules of water vapour diffuse more rapidly through air in conditions of low atmospheric pressure characterising high altitude environments than in atmospheric conditions of low altitudes⁸. Therefore, as a working hypothesis, we predicted that permeability of eggshells to water vapour is lower in eggs of birds breeding at high altitudes than in eggs of conspecifics breeding at low altitudes, thereby compensating for the increased diffusivity of water vapour in air at high altitudes.

During June 1976, eggs of barn swallows (*Hirundo rustica*) were collected from nests at nine localities along an altitudinal gradient running from the town of Crook in the South Platte River Valley of northeastern Colorado (elevation 1132 m), to South Park in the mountains of central Colorado (elevation 2883 m). The diffusivity of water vapour in air is approximately 25% higher at South Park than at low elevations in northeastern Colorado. Only one egg was taken from each nest because eggs of a single female are likely to have similar permeabilities to water vapour⁹.

Following transport of the eggs to our laboratory at Colorado State University, the eggs were placed randomly in vented desiccators above flakes of potassium hydroxide to maintain a dry atmosphere¹. The desiccators were held in a constant temperature cabinet at 25 °C, a temperature too low for embryogenesis to proceed¹⁰. Each egg was weighed six times on an analytical balance at approximately 24-h intervals, and the rate of weight loss—which we attribute largely to the escape of water vapour—was computed by a linear regression procedure. The volume of each egg was then measured by displacement of water, and its surface area calculated using the equation of Paganelli, Olszowska and Ar¹¹.

Assuming that the vapour pressure at inner surfaces of eggshells was 23.76 torr (that is, the vapour pressure of pure

water at 25 °C)^{12,13}, the conductance of eggs to water vapour was calculated from the formula

$$G_{H_2O} = M_{H_2O} / \Delta P_{H_2O}$$

where G_{H_2O} is the conductance of an egg to water vapour ($\mu\text{g d}^{-1} \text{ torr}^{-1}$), M_{H_2O} is the rate of water loss ($\mu\text{g d}^{-1}$), and ΔP_{H_2O} is the gradient in vapour pressure across the eggshell (torr). All values were corrected to sea-level pressure (ref. 8).

A regression analysis of variance (Table 1) revealed a linear decline in conductance of barn swallow eggs to water vapour with increasing altitude of the nesting site (Fig. 1). This reduction in water-vapour conductance of swallow eggs from montane localities is similar to that reported earlier for eggs of domestic fowl maintained for several generations at a high altitude¹⁴, but differs from the latter in an important respect. Reduced conductance of eggs of domestic fowl at high altitude just compensates for the increased diffusivity of water vapour,

Table 1 Regression analysis of variance of conductances of barn swallow eggs to water vapour in relation to altitude of the nesting sites

Source of variation	Sum of squares	d.f.	Mean squares	F-ratio	P
Linear regression	183,990	1	183,990	13.07	<0.005
Deviations from linearity	114,049	7	16,293	1.16	>0.25
Error	351,886	25	14,075		
Total	649,925	33			

This analysis indicates that a linear model is adequate to describe geographic variation in conductances of eggs to water vapour, and that the slope of the line is significantly different from zero.

resulting in total water losses from eggs during incubation that are similar to those experienced by eggs from low altitudes¹⁴. In contrast, the adaptive reduction in conductance of swallow eggs with increasing altitude of the nesting site is greater than that required simply to compensate for the increased diffusivity of water vapour at high altitude. For example, whereas a 17% reduction in conductance of swallow eggs would just compensate for the increased diffusivity of water vapour at 3000 m as compared to 1500 m, the observed reduction in conductance between these elevations is 59%.

Adaptation of barn swallows to high altitudes differs from

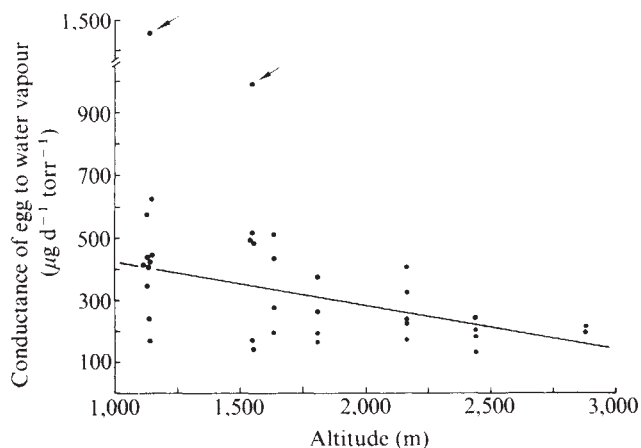


Fig. 1 Linear regression of conductances of barn swallow eggs to water vapour (corrected to sea-level pressure) against altitude of the nesting sites ($y = 564 - 0.140x$). The two extraordinarily high values identified with arrows represent eggs that probably suffered hair-line cracks during transport, and these values were not included in the analysis of variance (Table 1). Bartlett's test indicates that we are justified in assuming homogeneity of sample variances ($\chi^2 = 9.65$; d.f. = 8; $P > 0.1$). The correlation coefficient for the regression line is 0.53.

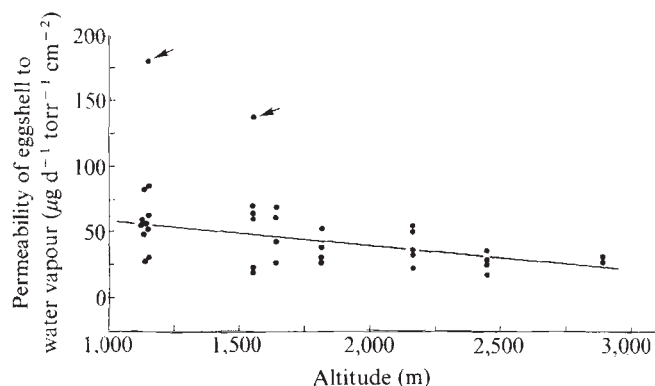


Fig. 2 Linear regression of permeabilities of barn swallow eggshells to water vapour against altitude of the nesting sites ($y = 75.18 - 0.0176x$). Permeability of each eggshell was computed by dividing the conductance of the egg to water vapour by the calculated value for surface area¹. The slope of the line is significantly different from zero ($F_{1,25} = 11.67$; $P < 0.005$), and the data do not depart significantly from linearity ($F_{7,25} = 1.39$; $P \approx 0.25$). The correlation coefficient for the regression line is 0.50. Arrows identify the statistical outliers to which reference was made in the caption to Fig. 1.

the example of domestic fowl in another important way. Adaptive reduction in conductance of hens' eggs at high altitude stems from reductions both in size of eggs (and therefore in surface area) and in permeability of eggshells to water vapour¹⁴. In contrast, eggs of barn swallows do not vary in size among the populations sampled in this investigation ($F_{8,27} = 1.49$; $P > 0.1$), and the entire decline in conductance with increasing elevation is attributable to a parallel decline in permeability of eggshells (Fig. 2). Thus, domestic fowl and barn swallows exhibit different adaptive strategies in their responses to reduced atmospheric pressure.

The observed pattern of altitudinal variation in permeability of barn swallow eggshells to water vapour has important implications pertaining to the comparative biology of montane and piedmont populations of the species. For instance, eggs of various bird species experience a loss of water during incubation amounting to 14–18% of the weight at laying^{3,7}. Assuming that eggs of barn swallows from montane and piedmont populations experience similar fractional weight losses prior to hatching, then incubation periods may be longer at high altitudes^{3,14}, or effective ventilation of nests may be increased at montane localities, thereby providing for lower vapour pressures in air adjacent to incubating eggs and for higher rates of water loss from eggs during natural incubation⁷.

The porosity of avian eggshells apparently is a heritable characteristic¹⁵. The size and number of pores through an eggshell is established at the time of shell formation⁹, and the permeability of the eggshell to water vapour remains constant throughout incubation¹⁶. When heritability of porosity is combined with a tendency for birds to return to the locality where they were born¹⁷, it is possible for natural selection to act on variation in porosity of eggshells to effect a differentiation of populations with respect to this characteristic¹⁸. Presumably, this explanation accounts for the origin and maintenance of the clinal pattern of variation observed by us for eggs of barn swallows.

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Virus-like particles in the nematode *Romanomermis culicivorax* (Mermithidae)

EARLY reports of virus infections of nematodes remain unconfirmed^{1,2}, although more recently virus-like particles were reported occurring in the intestinal cells of a plant-parasitic nematode³. The only confirmed reports of viruses found inside nematodes are plant viruses which occur in the digestive tracts of their nematode vectors⁴. We report here the presence of virus-like particles in cells of the infective stages of the mosquito parasitic nematode, *Romanomermis culicivorax*.

Cultures of *R. culicivorax* Ross and Smith (*Reesimermis nielsenii* Tsai and Grundmann) had been cultured *in vivo* in larvae of the mosquito *Culex pipiens quinquefasciatus* Say.

Cultures containing eggs of the nematode were flooded with water and yielded preparasitic (infective stage) juveniles that were prefixed for 1 h in phosphate-buffered 1% glutaraldehyde then transferred to a 1% solution of osmium tetroxide. They were then placed in agar blocks, dehydrated in an ethanol series and double embedded in Araldite. Sections made on a Porter–Blum microtome were stained with uranyl acetate and examined with a Philips EM 300 electron microscope.

Each of 10 preparasitic juveniles of *R. culicivorax* examined contained virus-like particles in the hypodermal cords, cells

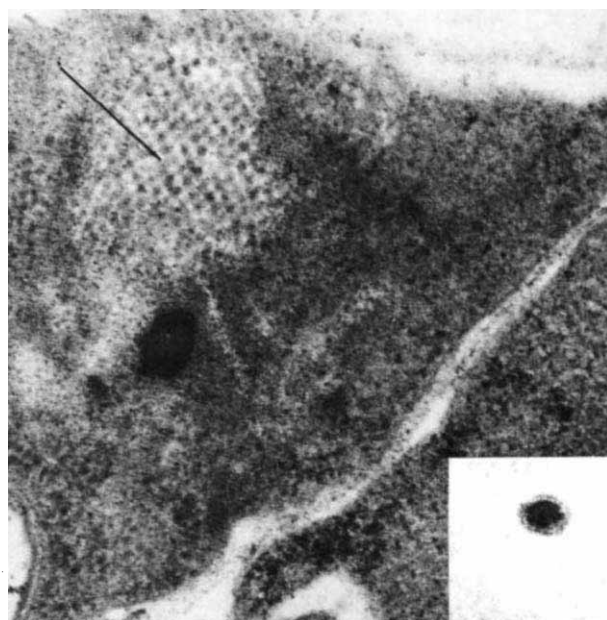


Fig. 1 Crystalline array of virus-like particles (arrow) in hypodermal cord of preparasitic juvenile of *R. culicivorax* ($\times 50,000$). Insert shows isolated virus-like particle with inner core and outer envelope ($\times 100,000$).

associated with the amphidial nerves, and spaces of the pseudocoelom. The particles were isometric, developed in the cytoplasm and those found outside the cell were enveloped (Fig. 1 insert). The diameter of the enveloped particles was approximately 50 nm while core (without envelope) measured 33 nm. In the host cells, the particles were either arranged in a crystalline array or randomly in the cell (Fig. 1). In those cases where development occurred in the hypodermal cords, damage to the latter was evident through cell disruption and lysis. Other stages of the nematode have not yet been examined for the presence of similar particles. The virus-like particles could be restricted to this or other nematodes or they could have been originally acquired through contact with mosquito larvae.

If the particles are infective, they would have to be transovarially transmitted in the nematode because the chances that they were acquired during nematode development in the egg or in the short time the preparasitic had emerged from the egg are very slight.

The fact that *R. culicivora* is being commercially produced for widescale release in gardens and the field clearly amplifies the importance of this discovery. Damage to the hypodermal cords by the virus-like particles could lower the efficiency of this nematode as a biological control agent.

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Nitrogenase activity of endophyte suspensions derived from root nodules of *Alnus*, *Hippophaë*, *Shepherdia* and *Myrica* spp.

STUDIES of nitrogenase of root nodule homogenates have focused chiefly on the *Rhizobium*-legume system. Little is known about the nitrogenase of actinomycete-like endophytes of root nodules on woody, non-leguminous plants. Although nitrogen fixation has been reported in suspensions of the endophyte from *Myrica cerifera*^{1,2}, the results were either of very low significance or irreproducible. In both cases dithionite and oxygen were added, which seems to be contradictory. Subsequent attempts to detect nitrogen fixation in non-leguminous nodule homogenates have been unsuccessful and investigations stopped at an early stage. We have found³, however, that reasonable levels of acetylene reduction by nodule homogenates of *Alnus glutinosa* can be measured if 0.3 M sucrose and 0.1 M dithionite are present during anaerobic homogenisation. The latter was essential because the nodule material contains large amounts of phenolic compounds, which after oxidation inhibit nitrogenase activity. While determining optimal conditions for the nitrogenase assay it was found that the reaction was ATP-dependent but that addition of an ATP-generating system (creatine phosphate (Cr~P)/creatine phosphokinase) decreased the activity during short-term experiments. This inhibitory effect was not found when the same ATP-generating system was added to bacteroid suspensions derived from leguminous nodules. We report here the seemingly aberrant behaviour of the added ATP-generating system, the applicability of our method to other actinomycete-woody plant symbioses, and the separation of actively acetylene-reducing endophyte material from nodule homogenates.

To get a better insight into the effect of an added ATP-generating system on the nitrogenase activity of endophyte

suspensions of *A. glutinosa*, the reaction was followed over an extended period. As an ATP-generating system we used phospho(enol) pyruvate (PEP) and pyruvate kinase (PK). Figure 1 shows that between 30 min and 8 h acetylene was reduced at equal rates when either ADP or ATP was added to nodule homogenates with PEP and PK. Because ADP only produced no nitrogenase activity, we concluded that the ATP-generating system can function in nodule homogenates of *A. glutinosa*. Within the first 2 h, however, the rate of acetylene reduction in complete systems was lower than that in the control. These results show that at least during the first 2 h of incubation there was no apparent inhibition of nitrogenase by ADP produced from ATP. This is in contrast to

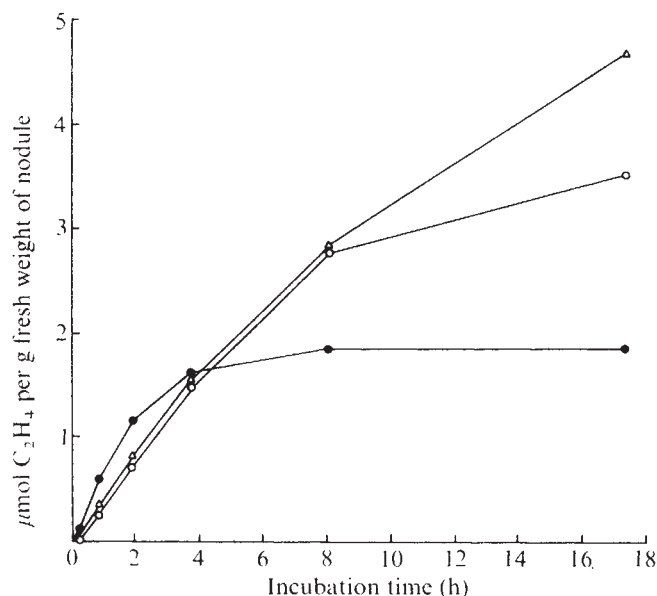


Fig. 1 Effect of PEP (10 mM) and PK (0.01 mg ml⁻¹) on acetylene reduction by nodule homogenate of *Alnus glutinosa*. General procedure for homogenisation, filtration and incubation as described previously³, with the following modifications: 2 min homogenisation at 31,000 r.p.m. in the presence of 100 mM dithionite. The 100-μm filtrate is referred to as the nodule homogenate. The assay mixture (2 ml) contained: 1 ml of filtrate (corresponding to 14 mg of fresh nodule tissue), 2% PVP, 0.15 M sucrose, 100 mM Na₂S₂O₄, 2.5 mM 1,4-dithioerythritol, 50 mM Tris-HCl (pH 8.0), and 5 mM MgCl₂. Where added, ATP and ADP were 5 mM. Δ, ATP, PEP and PK; ○, ADP, PEP and PK; ●, ATP.

results obtained with bacteroid suspensions derived from leguminous nodules^{4,5}.

It was not clear why addition of the ATP-generating system initially inhibited nitrogenase activity (Fig. 1), and so the effect of the high-energy phosphate donor was tested. Table 1 shows that

Table 1 Effect of creatine phosphate (Cr~P) and phospho(enol) pyruvate (PEP) on acetylene reduction by nodule homogenates of *Alnus glutinosa*

PEP (mM)	Cr~P (mM)	Acetylene reduction (μmol C ₂ H ₄ per h)¶
0	0	1.20*
0	1	1.11*,†
0	5	1.00†,‡
0	10	0.82‡,§
1	0	0.93‡
5	0	0.73§
10	0	0.65§

Procedures for homogenisation and incubation are described in Fig. 1. All incubation mixtures contained 5 mM ATP. Means followed by the same reference mark are not significantly different by Tukey's ω procedure at 1% level.

¶ Calculated per g fresh weight of nodule.

Table 2 Acetylene reduction by excised nodules and by nodule homogenates of *Alnus*, *Hippophaë*, *Shepherdia* and *Myrica* spp.

	Na ₂ S ₂ O ₄ during incubation (mM)	Incubation period (min)	Acetylene reduction ($\mu\text{mol C}_2\text{H}_4$ per h)§			
			<i>A. glutinosa</i>	<i>H. rhamnoides</i>	<i>S. canadensis</i>	<i>M. gale</i>
Intact nodules*	—	0–6	20.41	11.22	—	0.24
		6–12	18.59	13.40	2.87†	1.39
Nodule† homogenate	25	0–20	4.04	2.51	0.97	0.40
	50	0–20	4.86	3.23	1.18	0.50
	100	0–20	5.19	3.13	1.13	0.60
	150	0–20	4.66	2.70	0.92	0.72

*The rate of acetylene reduction by excised nodules was measured after 6 and 12 min of incubation in 10% C₂H₂ in air at 22 °C. *Alnus* and *Hippophaë* spp. were grown in the greenhouse, *Shepherdia* and *Myrica* spp. came from the field.

†Methods as described in Fig. 1, with the exception that the concentrations of both ATP and Mg²⁺ were 15 mM. The reaction was stopped after 20 min by adding 0.5 ml 6 N HCl.

‡Incubation period 3–12 min.

§Calculated per g fresh weight of nodule.

both Cr~P and PEP inhibited acetylene reduction. The concentrations used were rather low compared with those given in the literature concerning ATP-generating systems for measuring *in vitro* activity of nitrogenase (38–50 mM Cr~P (refs 4, 5)). Higher concentrations inhibited the activity even more. The nature of this inhibition is not yet known.

Since addition of an ATP-generating system had no positive effect in short-term experiments we tried to enhance acetylene reduction with increasing ATP and Mg²⁺ concentrations (Fig. 2). Maximum levels of activity were achieved at 10 mM ATP (ATP/Mg²⁺ = 1). There was no significant difference (0.01 level

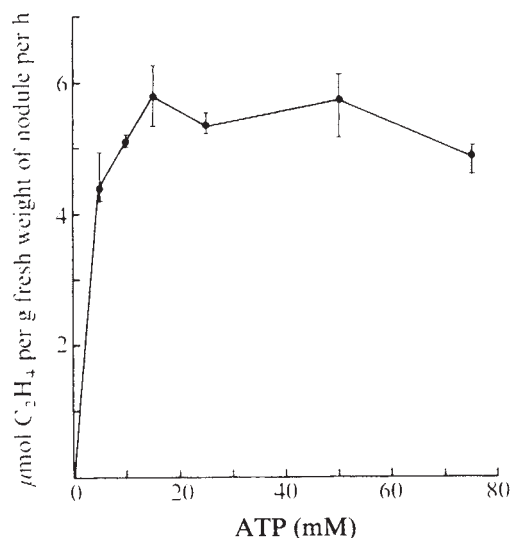


Fig. 2 Effect of ATP concentration on acetylene reduction by nodule homogenate of *Alnus glutinosa*. ATP/Mg²⁺ = 1. Vertical brackets denote the extreme range of four replicates.

of significance) in activity when the concentrations were increased two or fivefold. The values for nitrogenase activity shown in Fig. 1 are considerably higher than those reported earlier³, when sub-optimal levels of ATP and Mg²⁺ were used.

Using a constant ATP concentration of 15 mM we tested the applicability of our homogenisation and incubation method to a series of actinomycete-plant symbioses. Because of the unexpectedly high optimum concentration of dithionite for *A. glutinosa*, we tried a range of concentrations. Table 2 shows that significant nitrogenase activities of nodule homogenates of various species can be demonstrated in this way and the optimum dithionite concentration seems to be different for these four types of nodules. The differences in recovery ($\pm 25\%$ for *Alnus* and *Hippophaë* spp., and $\pm 40\%$ for the other two species) result from the differences in activity of the detached nodules. This conclusion

is based on observations with alders.

To study the metabolic processes in the endophyte, it is necessary to separate the intact endophyte from the host. The most prominent structures of the endophyte are the vesicle clusters. As these clusters show a strong tetrazolium-reducing activity it has been suggested⁶ that they might also be involved in nitrogen fixation. We have developed a method to collect and purify these structures by anaerobic filtration of nodule homogenates^{3,6}.

In preliminary experiments, however, acetylene reduction by the fraction containing the vesicle clusters was less than 5% of the activity of the nodule homogenate³. We have now found that the homogenisation procedure used previously was too rough and too many vesicle clusters were disintegrated. Using a milder procedure (Virtis 45 homogeniser, 4 min at 3,000 r.p.m.) more vesicle clusters remained intact. Fractionation was improved further by collecting the vesicle clusters on a 10- μm filter. By homogenising nodules of *A. glutinosa* in this way, an activity of 2.59 μmol of C₂H₄ (calculated per g fresh weight of nodule per h, being 60% of the activity of the nodule homogenate) was obtained by the resuspended residue from the 10- μm filter. The 10- μm filtrate, consisting of hyphal fragments, disrupted vesicle clusters and plant cell debris, contained 29% of the activity of the homogenate, which was all particle bound. The remaining 11% of the activity was lost during the procedure. Results were similar with *Hippophaë rhamnoides*, although the activity of the resuspended residue from the 10- μm filter was only 35% of the activity of the nodule homogenate. We think that the high recovery of nitrogenase activity in the fraction containing the vesicle clusters is an additional indication that nitrogenase is indeed located in the vesicle clusters.

As it is now possible to collect metabolically active vesicle clusters on a large scale, attention will be focused on these structures. Their relatively high nitrogenase activity *in vitro* opens the way for further physiological and biochemical studies.

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Adenylate cyclase activity oscillations as signals for cell aggregation in *Dictyostelium discoideum*

As well as acting as a chemotactic factor¹ cyclic AMP stimulates cells of *Dictyostelium discoideum* to differentiate after the end of growth into aggregation-competent cells. These differentiation signals have a temporal pattern: pulses are efficient signals, whereas continuous administration of cyclic AMP may even have an adverse effect^{2,3}. *D. discoideum* cells can release cyclic AMP spontaneously as reiterated pulses⁴, and also release cyclic AMP in response to extraneous cyclic AMP pulses⁵⁻⁷, which is important for the transmission of signals in aggregation territories in form of propagated waves⁸⁻¹². The pulsatile release of cyclic AMP into the extracellular space is preceded by a sharp (about 10-fold) increase of its intracellular concentration within 1 min (ref. 4). This indicates that the oscillating variable is not only transport but also net synthesis of cyclic AMP. The periodic rise of the intracellular cyclic AMP concentration could be due to periodic activation of adenylate cyclase, to inhibition of phosphodiesterase, or to the oscillatory control of both enzymes. We present here evidence for sustained oscillations of the adenylate cyclase activity in signalling cells. No concomitant changes of the ATP concentration, the substrate of adenylate cyclase, were found.

Periodic activities can be recorded in *D. discoideum* by measuring the light scattering of cell suspensions¹³. These measurements provide reference points for correlating the adenylate cyclase activities in different phases of a cycle with changes in cyclic AMP concentration in living cells.

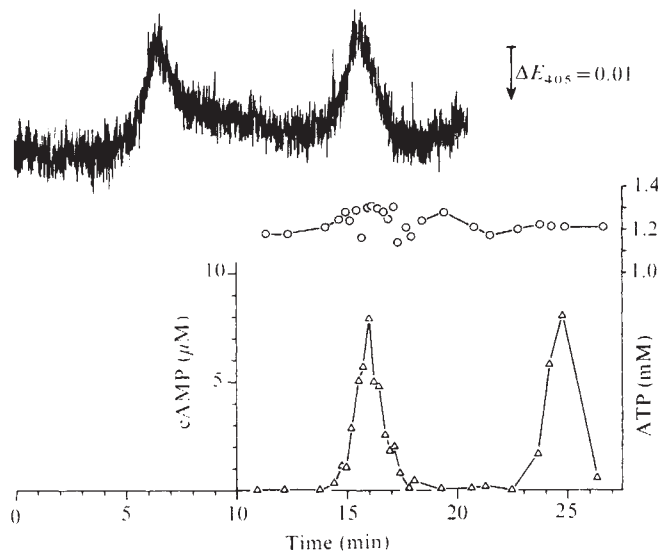


Fig. 1 Cyclic AMP (Δ) and ATP ($\circ$) concentrations in periodically signalling cells. Changes of light scattering (top) indicate periodic activities in the cell suspension. At intervals of 9 min the total cyclic AMP increases up to a sharp peak, whereas ATP in the same cells does not show significant oscillations. Because in the extracellular space cyclic AMP is rapidly degraded by phosphodiesterase²², the cyclic AMP is predominantly intracellular and has been calculated like ATP per volume densely packed cells (left and right ordinates). Cyclic AMP was assayed by Gilman's²³ method after the addition of 5% TCA to 50 μ l-samples of the cell suspension, and handling of the material as described by Gerisch and Wick⁴. ATP was extracted from suspended cells with 3% perchloric acid and determined by the bioluminescence assay²⁴. Cells of the axenic strain Ax-2, clone 206, were used 5 h after their transfer from growth medium into non-nutrient buffer. The cell density was 5×10^7 per ml, other conditions and light scattering measurements were as described before¹³.

Spikes of decreased light scattering coincide with peak concentrations of cyclic AMP⁴. ATP concentration, measured simultaneously, did not oscillate detectably (Fig. 1): the steady-state ATP concentration was 150-fold higher than the cyclic AMP peaks. The depletion of the ATP pool of the cells due to cyclic AMP synthesis is therefore negligible, and should be rapidly compensated by respiratory control. The possibility remains that ATP, being compartmentalised, oscillates with larger amplitudes at the location of adenylate cyclase than it does on average in the whole cell.

The cyclic AMP concentration rises for not longer than 2 min in each cycle (Fig. 1), indicating similarly short phases of adenylate cyclase activation. Using the method of Salomon *et al.*¹⁴, we reduced the assay time to 15 s, facilitating the detection of periodic short-term increases of the adenylate cyclase activity (Fig. 2). During the enzyme assay any regulatory influences due to varying concentrations of either the substrate or the product in different phases of a cycle were eliminated by adding both ATP and cyclic AMP in sufficiently high concentrations to cell homogenates.

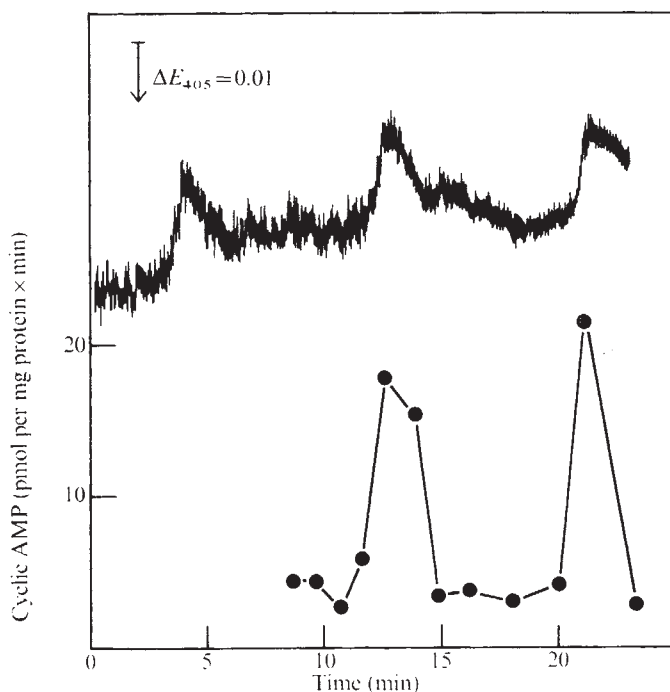


Fig. 2 Oscillations of adenylate cyclase activity in a cell suspension. Samples for the adenylate cyclase assay (bottom) were taken during continuous recording of light scattering (top). The cells were cultivated and prepared as described before¹³ and used 4 h after transfer from growth medium into non-nutrient buffer. The strain was Ax-2 clone 206, and the cell density 1×10^8 ml⁻¹. The adenylate cyclase assay was performed according to Salomon *et al.*¹⁴ after sonication of the cells for 5 s as described by Roos and Gerisch¹⁶. The ATP concentration was 0.18 mM, the cyclic AMP 1 mM, and homogenate protein 1 mg ml⁻¹.

High enzyme activities in homogenates, and cyclic AMP peaks in intact cells were seen during the same part of a cycle, as indicated by the coincidence with the spikes of decreased light scattering in cell suspensions (Figs 1 and 2). In each period the enzyme stayed activated for 2–3 min, as expected from the length of time during which the cyclic AMP concentration rises in intact cells. The adenylate cyclase then returned to basal activities (Fig. 2), in accord with the sharp fall of the cyclic AMP concentration in living cells (Fig. 1). The latter certainly results from deactivation of adenylate cyclase, from cyclic AMP transport into the extracellular medium⁴, and perhaps to a minor extent from the action of intracellular phosphodiesterase.

In homogenates the activated state of the adenylate cyclase decayed within 1 min (Fig. 4). It remains to be seen

whether the mechanism of deactivation is the same in the homogenate as in intact cells. It is obvious, however, that degradation of adenylate cyclase is not the reason for the decay of its peak activities in homogenates, since the basal activity remained almost constant for a longer period (Fig. 4).

From measurements on 6 cycles a mean basal activity of 4.2 ± 0.8 pmol cyclic AMP per mg protein per min was obtained, and a mean activation during pulse formation by the factor 7.1 ± 1.6 (Fig. 3). In the best cases the amplification factor was 10, and the peak activities were 40–50 pmol per mg protein per min. These adenylate cyclase activities obtained in cell homogenates are comparable with the rates of cyclic AMP increase measured in living cells. A maximal rate of about 70 pmol per mg protein per min can be calculated from Fig. 1. Previously reported values have been not higher than 300 pmol per mg protein per ml (ref. 15).

What are the mechanisms of adenylate cyclase regulation? In cells which have not yet started to oscillate, adenylate cyclase is transiently activated by pulses of extraneous cyclic AMP which acts on cell surface receptors¹⁶. In spontaneously oscillating cells a positive feedback loop may connect cyclic AMP release, the activation of cell surface receptors, and the activation of adenylate cyclase. In fact, precocious pulses and phase shifts induced by small pulses of extraneous cyclic AMP, demonstrate a connection between the receptors and the system that periodically activates adenylate cyclase¹³. This focuses attention on the regulatory pathway from the cell surface receptors to adenylate cyclase. One possibility is that upon receptor activation a soluble intracellular effector is formed, alternatively the enzyme might undergo covalent modification, for example by phosphorylation. Calcium, which at high concentrations inhibits adenylate cyclase¹⁷ is a possible soluble effector. It seems unlikely, however, that periodic calcium sequestration is the mechanism which causes the enzyme activity to oscillate. Calcium 8.5 mM inhibited the adenylate cyclase in both its activated and non-activated

state. EGTA 3 mM did not prevent the decay of the activated state in cell homogenates. Furthermore, when added to a homogenate with basal adenylate cyclase activity, EGTA did not activate the enzyme. An earlier report¹⁸ on the activation of adenylate cyclase by 5'-AMP has not been confirmed by recent work^{16,17}.

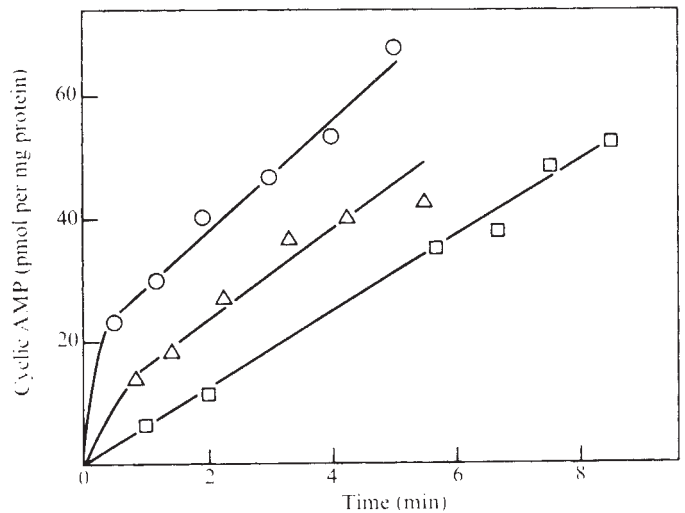


Fig. 4 Cyclic AMP synthesis in homogenates prepared at a peak of adenylate cyclase activity (○), at the phase 3 min before (□) and in a 1:1 mixture of both (△). The initial rate of synthesis in the mixture was the average of the basal activity found in the non-activated state, and of the high initial rate characteristic of the activated state. The activity remaining after the decay of the activated state was similar to the basal activity. Methods were as for Fig. 2, except that the concentration of ATP was 0.5 mM, and of protein 1.8 mg ml⁻¹.

To test for any soluble effector, homogenates containing activated and non-activated adenylate cyclase were mixed. The enzyme activity in such a mixture was the average of the activities in the individual homogenates (Fig. 4). This renders the presence of a soluble activator or inhibitor in homogenates from either one phase of the cycle unlikely, and favours the possibility of reversible covalent modification of adenylate cyclase.

In summary, the periodic activation of an enzyme, adenylate cyclase, is a key event in the generation of intercellular signals in *D. discoideum*. The finding that the total cellular ATP does not oscillate except within the limits of experimental error, poses certain restrictions on an allosteric model of adenylate cyclase oscillations in which substrate oscillations are essential¹⁹. The *D. discoideum* oscillator differs from chemical and biochemical oscillating systems, like the Belousov-Zhabotinsky reaction²⁰ or glycolysis in yeast extracts²¹, in that it is composed of two compartments separated and connected by the cell membrane. In these compartments cyclic AMP oscillates with different amplitude and phase¹. Cyclic AMP is produced intracellularly, is then released into the extracellular space, from where it feeds back to its own synthesis via cell surface receptors. This opens the possibility that the cell membrane constitutes an integral part of the oscillator, functioning both in cyclic AMP transport from the inside to the outside, and as a signal transducing element in the opposite direction.

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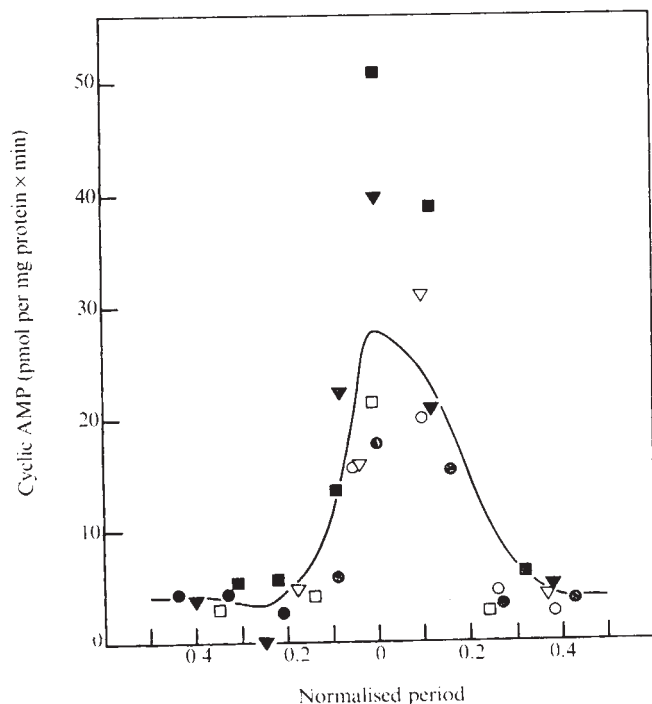


Fig. 3 Time course and amplitude of adenylate cyclase activation as the average (smooth curve) from 6 spontaneous pulses (symbols). The mean period was 7.8 ± 0.7 min. The periods were normalised and adjusted to the peaks of decreased light scattering (zero time). Methods were as in Fig. 2. Cells of clones 206 or 214 of strain Ax-2 were used at 3–4 h after replacement of the growth medium by non-nutrient buffer.

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Bacterial tumble regulator may be inactivated by methylation

KORT *et al.*¹ have described the methylation of a membrane protein which seems to be involved in bacterial chemotaxis: they show that addition of attractants affects the methylation, that the source of the methyl group is methionine, a compound required for chemotaxis, and that certain non-chemotactic mutants fail to carry out the methylation reaction. There is no relationship, however, between the amount of methylation and the tumble frequency, and the exact role of this methylation, therefore, remains mysterious. Control of the tumbling process is essential for chemotaxis; peritrichous bacteria swim in short straight-line runs that are separated by abrupt changes of direction (tumbles). In the presence of attractants, however, the tumbling frequency either decreases or increases as the bacterium swims up or down the concentration gradient, respectively². Runs are due to counter-clockwise rotation of the flagella, whereas clockwise rotation causes tumbling³. Aswad and Koshland⁴ have proposed the existence of a "tumble-regulating substance" that would control the direction of the rotation: clockwise rotation of the flagella (and therefore tumbling of the bacteria) occurring when the concentration of this regulating substance falls below a certain threshold. The same authors have suggested that *S*-adenosyl-methionine, the product of the reaction of one molecule of methionine with one molecule of ATP⁵, might be necessary for the degradation of the tumble regulator. I propose that the methylated protein described by Kort *et al.*¹ may be the inactivated tumble regulator.

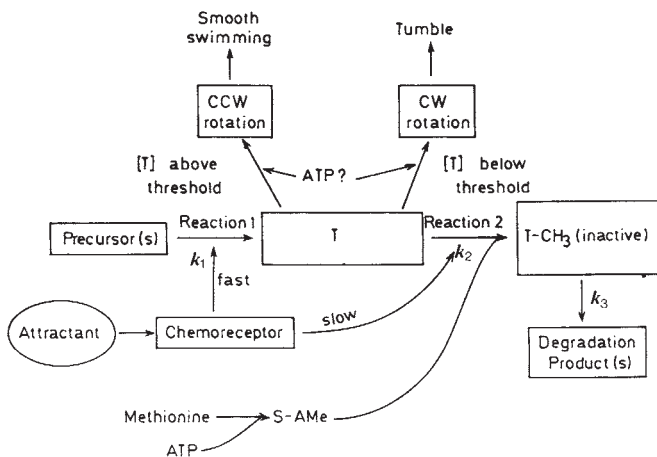


Fig. 1 Proposed scheme for inactivating tumble regulator. T, tumble-regulating protein; T-CH₃, methylated protein; S-AMe, *S*-adenosyl-methionine; CCW, counterclockwise; CW, clockwise. ATP?, ATP may be necessary for the direct control of the direction of flagella rotation².

Table 1 Effect of mutational changes of k_1 , k_2 and k_3 on swimming behaviour and methylation

Mutation	[T]	Mobility	[T-CH ₃]
$k_2 \uparrow$	$\uparrow$	Smooth	$\uparrow$
$k_1 \uparrow$	$\downarrow$	Tumbling	$\downarrow$
$k_3 \uparrow$	$\uparrow$	Smooth	$\downarrow$
$k_1 \uparrow$	$\downarrow$	Tumbling	$\uparrow$
$k_2 \uparrow$	$\uparrow$	Smooth	$\downarrow$
$k_3 \uparrow$	$\downarrow$	Tumbling	$\uparrow$
k_1/k_2 unch.	—	Normal	—
$k_1/k_2 \uparrow$	$\uparrow$	Smooth	$\downarrow$
$k_1/k_2 \downarrow$	$\downarrow$	Tumbling	$\uparrow$
k_1/k_2 unch.	—	Normal	—
$k_1/k_2 \uparrow$	$\uparrow$	Smooth	$\downarrow$
$k_1/k_2 \downarrow$	$\downarrow$	Tumbling	$\uparrow$
$k_2 \downarrow, k_3 \downarrow$	$\uparrow$	Smooth	$\downarrow$
$k_2 \downarrow, k_3 \uparrow$	$\downarrow$	Tumbling	$\uparrow$

T, tumble regulator; T-CH₃, methylated tumble regulator; Pr, precursor(s); S-AMe, *S*-adenosyl-methionine; unch., unchanged. At the steady state

$$[T] = k_1[Pr]/k_2[S-AMe] \quad [T-CH_3] = k_1[Pr]/k_3$$

Mutations implying other combinations of constant variations can be visualised as well as mutations influencing the concentrations of S-AMe and of the precursor(s).

In my proposed scheme, I have made use of the mechanism described by Macnab and Koshland⁶ and by Springer *et al.*⁷—that combination of an attractant with the chemoreceptor increases k_1 rapidly and k_2 slowly. This causes a transient increase in the concentration of the tumble regulator and, consequently, a transient repression of the clockwise rotation of the flagella (and thus of the tumbles).

My general scheme offers an explanation for the following experimental facts. The effect of methionine starvation on methionine auxotrophs^{1,4,7,8} or of the addition of arsenate to wild strains⁹ is that no *S*-adenosyl-methionine is formed: reaction 2 does not take place, protein T accumulates and the bacteria do not tumble. Accordingly, no methylated protein is formed and no chemotaxis can take place, which is in agreement with experimental data.

Variation of the constants k_1 , k_2 and k_3 may explain the occurrence of the various types of mutants described by Kort *et al.*¹ (see Table 1). Tumbling mutants with increased methylation and smooth-swimming mutants with decreased methylation are the most difficult to account for, as one must assume the concomitant variation of two of the three constants involved.

As a consequence of the increase in both k_1 and k_2 , induced by the addition of an attractant^{6,7}, the concentration of the methylated protein also increases, as observed by Kort *et al.*¹. The observation, however, that, within 10 min, the concentration of the methylated protein returns to its steady-state level, remains unexplained.

If my proposed idea is true, that methylation of the tumble regulator by *S*-adenosyl-methionine causes its inactivation, then it seems that the same principle has been utilised much later in evolution, since it is known that adrenaline and noradrenaline are inactivated by catechol-*O*-methyl transferase, an enzyme which utilises *S*-adenosyl-methionine as a methyl donor^{10,11}.

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Mucosal penetrability enhanced by serum-derived antibodies

ALTERED mucosal penetrability for food constituents and microbial components probably underlies the pathogenesis of a variety of diseases occurring in mucous membranes and distant sites such as liver and joints¹. The secretory immune system seems to be of major importance in mucosal exclusion of foreign material and is stimulated by topical antigens². Conversely, parenteral immunisation almost exclusively gives rise to circulating antibodies which are chiefly of the IgG class. IgG is not a secretory immunoglobulin in man, monkey, rabbit and guinea pig³, and contradictory information has been published about the effect of parenteral immunisation on the absorption of antigen through mucous membranes. Moreover, a possible influence on the concurrent mucosal penetration of unrelated macromolecules has not been considered previously. We report here that although serum-derived antibody retards the penetration of corresponding antigen, it nevertheless causes impairment of the mucosal barrier. Induction of IgG antibodies may thus cause hazardous side effects by altering mucosal penetrability.

Mucosal specimens devoid of a secretory immune system were obtained from the lingual frenum of 16 adult Belted Dutch rabbits, 8 of which had been immunised subcutaneously over a period of 2-3 months with a total of five 3-mg doses of human albumin emulsified in complete Freund's adjuvant. This schedule resulted in high levels of precipitating serum antibody (Table 1), which by immunoelectrophoresis was shown to be located chiefly in the IgG fraction. The excised membranes were rinsed in saline and mounted in diffusion chambers⁴ with the oral side exposed to 1 ml tissue culture medium containing 30 mg human albumin and 30 mg human transferrin, and with the basal side exposed to 200 μ l medium alone. After incubation for 2 h in a humid atmosphere of CO₂ in air at 37 °C, the basal concentrations of albumin and transferrin were measured. In preliminary experiments this was

attempted by isotope labelling which, however, was found to be unreliable⁵. Since radioimmunoassay has also been found unreliable in similar experiments⁶, the two components were quantified by single radial immunodiffusion performed essentially as described before⁷. To obtain sufficient sensitivity (detection limit of about 0.25 μ g ml⁻¹), specific rabbit antisera were used at final dilutions of 1:300 (anti-albumin) and 1:160 (anti-transferrin) in the Agarose gel, and the 5- μ l sample wells were refilled three times. The precipitin rings were intensified by overlaying the gel with filter paper soaked in 1:3 dilution of goat antiserum to rabbit IgG.

Table 1 shows that the control membranes discriminated between albumin and transferrin, according to the difference in molecular weights (69,000 and 90,000), as reported for other biological membranes *in vivo*⁸. This held true even for the specimen showing exceptionally high penetrability. Its defect was probably restricted to an epithelial damage, since penetration studies with rabbit intestinal mucosa have indicated that molecular selection takes place at the basement membrane whereas the epithelial layer is solely rate limiting⁹.

The penetration of albumin through membranes from immunised animals was reduced whereas the concurrent penetration of transferrin was enhanced (Table 1). The former result supports previous experiments performed with intestinal and oral mucosa of parenterally immunised animals^{10,11} and may be ascribed to immune complex formation. Binding of antigen to antibody in the mucus covering the intestinal surface has been suggested¹², whereas for the oral mucosa (gingival crevicular epithelium) ultrastructural indication of immune complex formation within the epithelial interstices has been presented¹¹. Ultrastructural studies of small intestinal mucosa, however, have indicated increased antigen absorption into epithelial cells after parenteral immunisation¹³.

Thus, the level at which serum-derived antibody retards mucosal antigen penetration has not been clearly defined. Since some IgG diffuses through epithelial interstices^{14,15}, immune complexes could be formed at the epithelial level, but also in the connective tissue. Exposure of epithelial surfaces to antigen in sensitised animals results in a rapid local emigration of neutrophils^{11,16}. Phagocytosis of immune complexes and degradation of antigen could therefore contribute to the apparently retarded mucosal antigen penetration. Moreover, release of enzymes from these cells probably explains the most important finding in our experiments—the enhanced penetration of an unrelated macromolecule. It is well known that neutrophil lysosomal enzymes can

Table 1 Effect of parenteral immunisation with albumin on the concurrent penetration of albumin and transferrin through rabbit oral mucosa

	Serum antibody*	Basal concentration as % of oral concentration†		Significance of difference‡
		Albumin	Transferrin	
Control animals		(0.0215)§	(0.0155)	
		0.0061	0.0034	
		0.0054	0.0018	
		0.0047	0.0035	
		0.0035	0.0008	
		0.0020	0.0009	
		0.0005	0.0005	
		0.0005	0.0005	
	Mean	0.0032	Mean 0.0016	P < 0.025
Immunised animals	8	(0.0037)	(0.0131)	
	2	0.0013	0.0025	
	32	0.0010	0.0055	
	16	0.0005	0.0076	
	32	0.0005	0.0032	
	16	0.0005	0.0020	
	16	0.0005	0.0018	
	16	0.0005	0.0016	
	Mean	0.0007	Mean 0.0035	P < 0.01
Significance of difference		P < 0.03	P < 0.05	

*Expressed as precipitating units based on tests against albumin (1 mg ml⁻¹) in gel diffusion.

†Each figure is an average of 2-4 measurements (detection limit 0.0008%, undetectable assigned 0.0005%).

‡Wilcoxon matched-pairs signed-ranks one-tailed test.

§Figures in parentheses excluded from group calculations because of the extreme deviations, apparently reflecting a membrane defect.

||Mann-Whitney one-tailed U-test.

affect basement membrane zones and the ground substance¹⁷, and mucosal penetrability may thereby be increased.

Several possible hazards of circulating antibodies resulting from vaccination have been discussed¹⁸. Our results point to one way in which IgG antibodies may jeopardise mucosal integrity since undue penetration of new antigens in turn may stimulate a broader IgG response and thus set up a vicious circle. The merit of the selective glandular secretion of IgA and IgM antibodies¹⁵ is probably to limit such a development. This must be taken into account when using vaccines in immunoprophylaxis and when the value of breast feeding is debated. In the neonatal period antigens are absorbed into the circulation in sufficient amounts to evoke a systemic IgG response in a great proportion of individuals, especially in those being artificially nourished¹⁹. This may predispose to untoward sensitisation of the child. In addition, our results substantiate one pathogenetic potential of local IgG-cell responses in mucosal disease¹⁴⁻¹⁵.

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Endplate current fluctuations reveal only one channel type at frog neuromuscular junction

SIGNAL transduction between nerve and muscle is mediated by a chemical process at the neuromuscular junction. In the nerve ending electrical activity causes the release of acetylcholine (ACh) into the synaptic cleft separating the nerve and muscle cells. ACh can bind to specific chemical receptors in the postjunctional muscle membrane; these receptors control a gating mechanism that can increase membrane conductance locally. Normal nervous activity triggers an action potential and contraction in twitch muscle fibres. At constant membrane potential the membrane conductance change induces a current that is carried largely by Na⁺ and K⁺ in frog¹, moving through ACh-gated membrane channels. There has been a controversy regarding the nature of the chemically gated endplate channels: are there kinetically-independent channels for Na⁺ and K⁺ or are these ions transported by the same channel? Maeno² initially proposed separate endplate channels, arguing that the two component decay of endplate potentials in procaine-treated cells resulted from a selective effect on Na⁺ channel kinetics. Kordas^{3,4} and Magleby and Stevens⁵ argued that Maeno's two-channel hypothesis could not account for the unique reversal potential observed or the monotonic voltage dependence of endplate current (EPC) decay. The analysis below of

iontophoretically-induced EPC fluctuations in procaine-treated and untreated preparations indicates that the ions which constitute the EPC are not carried by kinetically distinguishable endplate channels.

Our conclusions are based on the following quantitative description of the EPC. Consider a population of identical, independently functioning endplate channels in voltage clamp conditions at membrane potential V . When ACh is iontophoretically applied an EPC, μ_1 , will flow in the open channels

$$\mu_1 = \gamma P N (V - V_r) \quad (1)$$

where γ is the mean conductance of one open channel, P is the probability that any single channel will open when the endplate is exposed to ACh, N is the total number of channels, and V_r is the reversal potential at which there is no net current. The EPC will fluctuate if the application is prolonged, because individual channel lifetimes are of the order of several milliseconds⁶. The fluctuations are caused by changes in the actual number of conducting channels reflecting the probabilistic nature of channel opening and closure⁷. The intensity of the fluctuations may be characterised by their variance, σ_1^2 , about the mean EPC

$$\sigma_1^2 = \gamma^2 P (1 - P) N (V - V_r)^2 \quad (2)$$

Note that P is a function of ACh concentration which is non-uniform over the extended frog endplate. A more complex derivation which takes this spatial dependence into account produces essentially these same relations.

If the EPC were carried in separate Na⁺ and K⁺ channels, similar treatment would give the mean and variance of the EPC as

$$\mu_{II} = \gamma_{Na} P_{Na} N_{Na} (V - V_{Na}) + \gamma_K P_K N_K (V - V_K) \quad (3)$$

$$\sigma_{II}^2 = \gamma_{Na}^2 P_{Na} (1 - P_{Na}) N_{Na} (V - V_{Na})^2 + \gamma_K^2 P_K (1 - P_K) N_K (V - V_K)^2 \quad (4)$$

Here the subscripted variables have meanings similar to those described above. The approximate equilibrium potentials of Na⁺ and K⁺ in frog muscle are $V_{Na} \approx +40$ mV, $V_K \approx -100$ mV. Their exact values are unimportant as long as it is noted that they clearly differ from the observed reversal potential, $V_r \approx 0$ mV.

The two schemes may be distinguished experimentally by measuring the EPC variance at the observed reversal potential. For the single channel scheme at $V = V_r$, the net EPC is zero because the net current in each channel is zero (equation (1)); likewise the variance is zero (equation (2)). For the two-channel scheme at $V = V_r$, the net EPC is zero because the inward Na⁺ current is equal but opposite to the outward K⁺ current (equation (3)); however, the variance is non-zero (equation (4)) since the Na⁺ and K⁺ terms are each positive and cannot cancel.

EPCs were generated in voltage-clamped muscle cells of m. cutaneous pectoris from the frog *Rana pipiens*. Nomarski and modulation contrast optics were used to observe the endplates, and ACh was applied by iontophoresis 10-20 μ m from the cell. Normal Ringer's solution contained 100 nM tetrodotoxin whereas procaine-Ringer's did not; composition (mM): NaCl, 117.2; KCl, 2.5; CaCl₂, 1.8; Na₂HPO₄, 2.16; NaH₂PO₄, 0.85 (pH 7.0). The induced EPCs were processed by a digital laboratory computer. The variance was estimated as the frequency integral of the mean spectral density of the digitised current fluctuations, taking care to exclude miniature EPCs. The methods were similar to those of Dionne and Stevens⁸ and Ruff⁹.

Figure 1 contains data from a cell which was not treated with local anaesthetic. The mean EPC and its variance are plotted as a function of membrane potential, and it is seen that the variance approaches zero at the measured V_r . Similar observations were made on 22 untreated cells and 7 cells in 3×10^{-4} M procaine from 13 different frogs. In all cases the variance at the reversal potential was less than the uncertainty in its measurement and was typically about 2 orders of magnitude below its value at -60 mV.

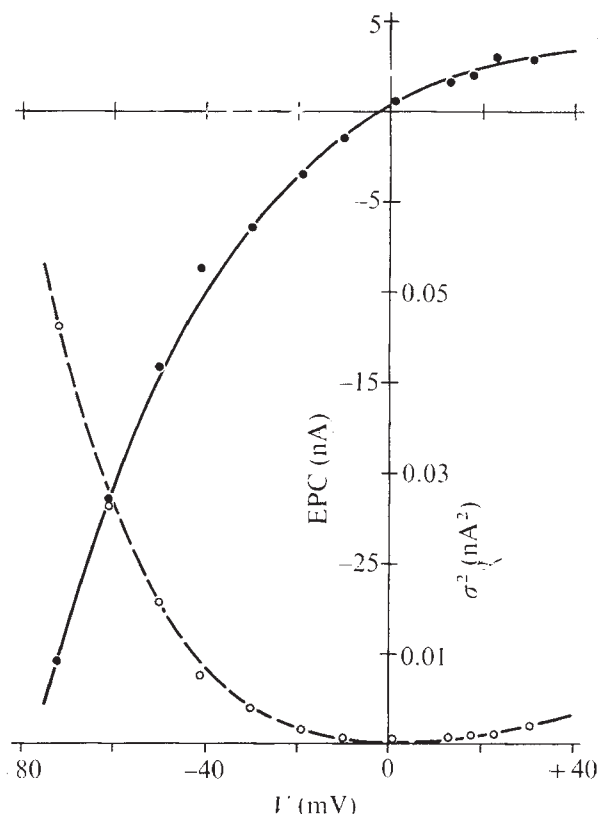
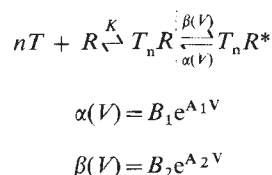


Fig. 1. Mean EPC and variance of EPC fluctuations about their mean are shown as a function of membrane potential, V . The smooth curve is equation (5) fitted to the EPC data to evaluate A , B and V_r . The values used here for the best fit are $A = -15.36 \text{ V}^{-1}$, $B = 144 \text{ nA}$ and $V_r = -2 \text{ mV}$. The same values are used to fit the voltage dependence of σ^2 with equation (6), the dashed curve. The single-channel conductance at -70 mV was 22 pS for this fibre. The quality of fit between theory and experiment in the variance measurements may be to some degree fortuitous. Most cells exhibited slightly greater measured variance at positive membrane potentials than predicted; however, to establish a $+30 \text{ mV}$ membrane potential, holding currents of several hundred nanoamperes were commonly required. Fluctuations in the holding current make accurate measurement of the few nanoampere EPC variations difficult. The data shown in this figure are from the cell with the best signal-to-noise ratio.

These data are inconsistent with the model in which Na^+ and K^+ pass through separately gated endplate channels. The two-channel model predicts that the variance of the EPC is non-zero at the reversal potential. Ruff⁹ also found that the variance of the EPC vanished at V_r for preparations exposed to the trimethyl lidocaine derivative QX-222, a different local anaesthetic.

The conclusion that a population of identical channels carries the EPC has been obtained by comparing measurement with theory at a single voltage, V_r . Equations (1) and (2) also provide descriptions of the functional dependence of EPC and its variance on voltage. Fitting these relations to the data, however, requires a more specific model of channel kinetics, because the probability of channel opening, P , is a function of voltage⁸.

As has been shown by others^{5,10,11}, normal channel operations may be represented by the following kinetic scheme.



where T represents agonist molecules, n of which bind to receptors R to produce at equilibrium an intermediate T_nR and an open channel T_nR^* . The probability of channel opening is voltage

dependent; for the case here of prolonged agonist exposure, the dependence is exponential and given by⁸

$$P = \beta C^n / \alpha K$$

$$= [C^n B_2 / K B_1] \exp(A_1 - A_2)V$$

where C is ACh concentration.

Thus, equations (1) and (2) may be rewritten to express their voltage dependence

$$\mu_1 = B(V - V_r)e^{AV} \quad (5)$$

$$\sigma_1^2 = \gamma B(V - V_r)^2 e^{AV} \quad (6)$$

In writing equation (6) it has been assumed that $P \ll 1$, an approximation which is accurate in the low agonist concentration limit that applies here. The voltage dependences of equations (5) and (6) are unchanged when a more detailed derivation includes the spatial non-uniformity of ACh concentration. Although equation (1) suggests that the EPC is proportional to voltage, we see in equation (5) a more complex relation as pointed out by Dionne and Stevens⁸. In addition, the variance (equation 6) is similarly affected by the voltage dependence of the probability of channel opening so that what seems to be a parabolic dependence in equation (2) is not so simple.

In Fig. 1 we have fitted equation (5) to the EPC-voltage data to evaluate A , B and V_r . These values were used in equation (6) which was drawn as the dashed curve through the variance measurements; the single-channel conductance γ was evaluated at -70 mV by taking the ratio of the ACh-induced conductance to its measured variance. The skewing of σ^2 by the voltage dependence of channel gating appears as a steepening of the parabolic behaviour for $V < V_r$ and a depression at $V > V_r$, and this behaviour is well described by equation (6). The ability to predict accurately the variance from measurements of the EPC is another indication of the essential correctness of the quantitative description of receptor kinetics which we have used.

Our description of endplate channel kinetics has been at two quantitative levels which contain certain basic assumptions in common. An elemental stochastic theory in which individual channels are represented as independent, two-state devices, either open or closed, leads us to conclude that Na^+ and K^+ currents at the endplate are simultaneously gated. This result follows because the EPC variance at the reversal potential is zero, contrary to the predictions of models assuming separately gated ion currents. The most parsimonious implication is that both Na^+ and K^+ share the same endplate channel. It should be emphasised that our results pertain to the gating of the chemically induced conductance change and do not address the question of whether Na^+ and K^+ have equal conductance in the open channel.

Extending this simple theory by incorporating a specific kinetic scheme for receptor function has allowed us to predict the voltage dependence of the EPC variance with coefficients determined from the mean EPC. The measured variance fits the predicted curve well over the range tested which supports the accuracy of the equations derived from the model. The kinetic model we used was selected because in the past it has accounted well for the general quantitative behaviour of the ACh-gated channel. Note that any kinetic scheme which yields quantitative predictions similar to ours would also be acceptable. Our conclusion that individual EPC ionic species are not separately gated is independent of the particular kinetics.

It may be useful to note the quantitative behaviour of our description over a voltage range more extensive than we have been able to test. If V could have been raised above $+100 \text{ mV}$ both the mean EPC and its variance are predicted by equations (5) and (6) to fall towards zero again. This phenomenon of EPC rectification is mediated largely by channel lifetime which approaches zero as membrane potential is increased. Since the dependence of channel lifetime on membrane potential is nonlinear, at positive voltages the shortened lifetime more than compensates for the increased driving potential on the current carrying ions and little or no EPC

can be induced. A similar mechanism may account for anomalous K^+ rectification in voltage sensitive channels in both cardiac and skeletal muscle.

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Selective activation of sympathetic ganglia in young spontaneously hypertensive rats

PROLONGED increase in peripheral sympathetic discharges producing vasoconstriction leads to enhanced synthesis of tyrosine hydroxylase (TH) and dopamine β -hydroxylase (DBH) in terminal adrenergic neurones^{1,2}. This is mediated by an increased activity of the preganglionic cholinergic nerves¹. Young spontaneously hypertensive rats (SHR) have been used as a prehypertensive model of essential hypertension to investigate whether such peripheral sympathetic neuronal activation initiates the increase of blood pressure. Study of the prehypertensive stage gives more information on the initiation of the blood pressure elevation than study of hypertensive animals. Circulating levels of noradrenaline, and DBH activity (as measures of peripheral sympathetic nerve system activity) were markedly elevated in young SHR (refs 3, 4), although contradictory results were reported for adrenomedullary activity³⁻⁵. In young SHR, adrenal levels of DBH, TH and phenylethanolamine *N*-methyl-transferase (PNMT) activities were lowered³, whereas other investigators^{4,5} found an elevation of adrenal DBH and PNMT activities. Transection of the splanchnic nerves markedly lowered blood pressure in young⁶ and adult SHR (refs 5, 7), and also reduced adrenal venous output of adrenaline in conscious young SHR (ref. 5). All

these findings suggest enhanced activity of the adrenal medulla and preganglionic cholinergic discharges to the adrenal medulla. We report here preganglionic nerve-dependent elevation of choline acetyltransferase (ChAc) and TH activities occurring specifically in the coeliac ganglia of young SHR.

In young SHR, the activity of preganglionic cholinergic nerve input to various sympathetic ganglia, superior cervical ganglia, stellate ganglia and coeliac ganglia, and adrenal glands was examined by measuring ChAc and acetylcholinesterase (AChE) activities as a measure of presynaptic neuronal activity⁸ and TH activity as an index of post-synaptic neuronal activity⁹ at the age of 4-5 weeks, when the blood pressure of SHR did not differ from normotensive controls. Normotensive animals of the Kyoto Wistar rats (KWR), from which SHRs were derived, were used as controls. Systolic blood pressures as measured by a tail plethysmographic method were 105 ± 11 mmHg (mean $\pm$ s.e.m.; $n=5$) for young KWR and 110 ± 7 mmHg ($n=5$) for young SHR, respectively. Ganglia and adrenals were removed and homogenised in pairs in 0.2 ml of ice-cold 0.005 M Tris buffer (pH 7.5) containing 0.1% Triton X-100. ChAc and AChE activities¹⁰ and TH activity¹¹ in the homogenates were determined radiometrically.

In young SHR, ChAc and TH activities of coeliac ganglia were significantly elevated as compared with those in young KWR. In the superior cervical ganglia, stellate ganglia and adrenals, however, there were no significant changes (Table 1). AChE activities in all ganglia and adrenals of young SHR did not differ significantly from those of KWR. Total protein contents of a pair of coeliac ganglia in young SHR (0.44 ± 0.03 mg, $n=5$) did not differ from those in KWR (0.41 ± 0.02 mg, $n=5$).

Table 2 shows the effect of sectioning the pre- and post-ganglionic nerves to the coeliac ganglion on its elevated ChAc and TH activities, and AChE activity. Bilateral preganglionic nerve transection markedly lowered the elevated ChAc activity to a level less than the ChAc activity of KWR 5 d later, but did not change TH and AChE activities. Bilateral post-ganglionic nerve section did not change ChAc, AChE and TH activities in the coeliac ganglion. Difference in the control values for ChAc and TH in both experiments (Tables 1 and 2) may be due to sham-operated stress as well as age difference (5 or 4 weeks). Since decentralisation of sympathetic ganglia removes synaptic input to the ganglia rather than nonspecific surgical damage¹², the present finding suggests that elevated ChAc levels are due to the enhanced ACh release and formation by the activation of preganglionic neuronal input to the coeliac ganglion of young SHR. The persistent elevation of ChAc activity in the coeliac ganglion even after the post-ganglionic axotomy negated the reflex of the innervating end-organ activity. Removal of sympathetic target organs, however, is known to prevent the normal maturation of ChAc in presynaptic endings during development¹³. Selective activation of the coeliac ganglion may be explained by an enhanced reactivity of the ganglia to pre-ganglionic nerve discharges rather than the regional elevation of nerve input. After treatment with nerve growth factor, the prevertebral coeliac

Table 1 Enzyme activities in sympathetic ganglia and adrenals of young spontaneously hypertensive (SHR) and Kyoto Wistar rats (KWR) at 5 weeks of age

Organ	Choline acetyltransferase (nmol ACh per h per pair)		Acetylcholinesterase (nmol acetate per h per pair)		Tyrosine hydroxylase (nmol CO ₂ per h per pair)	
	KWR	SHR	KWR	SHR	KWR	SHR
Superior cervical ganglia	3.84 ± 0.41	3.52 ± 0.48	23.6 ± 1.5	22.0 ± 0.72	0.115 ± 0.009	0.102 ± 0.001
Stellate ganglia	2.86 ± 0.26	2.98 ± 0.31	21.7 ± 1.2	22.8 ± 0.77	0.081 ± 0.009	0.094 ± 0.016
Coeliac ganglia	2.88 ± 0.51	$4.16 \pm 0.6^*$	21.3 ± 0.9	19.6 ± 0.45	0.180 ± 0.019	$0.252 \pm 0.021^*$
Adrenals	11.8 ± 0.8	13.6 ± 1.1	62.6 ± 3.2	66.1 ± 3.2	0.842 ± 0.195	1.14 ± 0.15

Values are mean $\pm$ s.e.m. for 5 animals at 5 weeks of age.

* $P < 0.05$ compared with KWR.

Table 2 Effects of pre- and post-ganglionic nerve transection of the bilateral coeliac ganglia on its elevated choline acetyltransferase activity in young SHR at 5 weeks of age

Group	Choline acetyltransferase (nmol ACh per h per pair)	Acetylcholinesterase (nmol acetate per h per pair)	Tyrosine hydroxylase (nmol CO ₂ per h per pair)
SHR, sham operated	3.27 ± 0.32	21.3 ± 2.3	0.167 ± 0.009
SHR, preganglionic axotomy	0.47 ± 0.08*	20.7 ± 1.4	0.152 ± 0.011
SHR, postganglionic axotomy	2.66 ± 0.33	19.8 ± 1.2	0.175 ± 0.019
KWR, sham operated	2.25 ± 0.15†	19.5 ± 0.8	0.134 ± 0.011†

Values are mean ± s.e.m. for 5 animals at 4 weeks of age. Axotomy was performed under ether anaesthesia 5 d before killing. Differences in control values for ChAc and TH when compared with Table 1 are probably due to the effects of age difference and laparotomy stress.

**P* < 0.01 compared with SHR controls.

†*P* < 0.05 compared with SHR controls.

ganglion shows a significantly more pronounced hypertrophy than the paravertebral superior cervical ganglion and stellate ganglion in young rats¹⁴. Our results indicate possible selective maturation of coeliac ganglia in young SHR. This is supported by findings that there is a rostro-caudal gradient in TH ontogeny in various sympathetic ganglia, for example, in maturation of the sixth lumbar ganglia at 2 months of age¹⁵ and in superior cervical ganglia at 14 d (ref. 16). Thus it suggests that in the coeliac ganglia, TH activity would not reach adult plateau values by 4–5 weeks of life, the age of young SHR in our experiments.

The observed activation of the coeliac ganglion in young SHR may enhance the release of noradrenaline and DBH levels into peripheral circulation from the coeliac ganglion-innervated arteries in splanchnic region and others. It is clear that in young, prehypertensive SHR, the selective activation of pre- and post-synaptic neuronal activities in coeliac ganglia initiates the elevation of blood pressure.

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Colour-coding properties of sustained and transient channels in human vision

WHEN the colour of a visual stimulus is repetitively alternated between say red and green, the percept evoked depends upon the temporal frequency of repetition. At very low frequencies (for example, 1–2 Hz), the repetitive change in colour is readily apparent. But at frequencies above about 10 Hz, no changes in colour are perceived; the stimulus in this case would appear a uniform yellow but would still seem to be flickering in brightness. Observations of this kind suggest that the colour of a stimulus may be analysed by a set of channels different from that analysing the brightness or flicker. The flicker or brightness channels respond to higher temporal frequencies of flicker than do the colour channels. This idea was confirmed by King-Smith¹ who has described how the sensitivity to coloured flashes of light changes with flash duration. He further described the subjective appearance of the flash, and concluded that a stimulus was not necessarily detected by the same channels as those that detected its colour. When the

flash was brief, the stimuli usually looked achromatic, except when the wavelength was below about 490 nm. The intensity of the flash had to be raised above the detection threshold before the colour could be perceived. At longer flash durations, the colour was apparent as soon as the flash was detectable. The channels signalling the colour of a stimulus have a longer 'integration time' than those giving an achromatic percept. This kind of experiment examines differences in the high temporal frequency behaviour of the two sets of channels. It is of interest to examine the low temporal frequency behaviour as well because recent neurophysiological investigations of the monkey's visual system make use of this behaviour in classifying neurones^{2–5}. Some neurones give a sustained response to prolonged presentation of a stationary stimulus; others respond only transiently to the stimulus onset. I show here that human colour channels give sustained responses while the flicker or brightness channels give transient responses.

The stimuli were disks (1 diameter) viewed against a white background (3.5 diameter). The background, viewed through a pellicle, was made by illuminating a diffusing screen with a tungsten halide bulb of unknown colour temperature. The luminance of the background was 50 cd m⁻² which gave a retinal illumination of about 1000 td (5 mm pupil). The light source for the 1 test stimuli was the P31 phosphor of an oscilloscope. This is not ideal as the phosphor emits little red light, so that the range of wavelengths examined is limited. Four test 'colours' were used: blue (Ilford 621; nominal peak 450 nm), green (Ilford 807; 530 nm), yellow (Ilford 626; 570 nm) and orange (Ilford 607; 590 nm). The widths of these filters at half maximum transmission are 30–80 nm.

Test stimuli were flashed on and off at intervals of 4 s while the background remained steadily illuminated. The observer adjusted the intensity of the test stimuli until he considered that they were only just visible. Eight threshold settings were made for each stimulus. In the first series of experiments, the observer attempted to detect an 8-ms flash of the stimulus superimposed on a subthreshold flash lasting 1 s. The test and subthreshold flashes were spatially co-extensive and were of the same colour. It has been argued⁶ that this technique examines the subliminal response of channels to the subthreshold long flash. Thus, if the subliminal response is transient, the sensitivity for the brief flash will be increased soon after the onset of the conditioning flash but will return to its control value after 100–200 ms, as the transient response subsides. But, if the subliminal response is sustained, the sensitivity will not return to its control value until after the end of the conditioning flash.

Figure 1a illustrates the results of such an experiment for three different colours. In all three cases the sensitivity to the test flash was increased when it appeared 16 ms after the onset of the conditioning flash. For green and orange stimuli, the sensitivity returned almost to normal when the test flash appeared 500 ms after the onset of the conditioning flash. The subliminal response to the long flash was transient. For the blue flash, however, the sensitivity remained high, suggesting a sustained subliminal response.

In confirmation of King-Smith's finding, the brief green and orange flashes appeared almost achromatic while the blue flash did appear a deep blue. The almost achromatic percept was of a silvery glow with perhaps a tint of the appropriate colour. No attempt was

made to show rigorously that the percepts evoked by the green and orange stimuli were indistinguishable.

Figure 1c shows the results of a similar experiment, but with test flashes lasting 250 ms. For both the green and orange stimuli, the sensitivity to the test flash remained higher than normal even when it was presented 500 ms after the onset of the long flash. The subliminal responses of the channels detecting the 250-ms flashes were sustained. Again, confirming King-Smith's observations, the test flashes (being long) appeared coloured.

These results suggest that if a narrow-band stimulus appears coloured, it is detected by sustained channels. If it seems achromatic, it is detected by transient channels. It might be expected that, if two stimuli seem to be of different colours, they would be detected by channels responding to different narrow bands of wavelength; the stimuli might be detected independently. Apparently-achromatic stimuli of different wavelength might be detected by one set of broad band channels; stimuli of different wavelength would summate.

Figure 2a illustrates an experiment to test this prediction. Two test stimuli of different colour were generated using two oscilloscopes viewed through a half-silvered mirror. In the upper part of the figure, a yellow flash was added to a simultaneous green flash. The relative intensities of the two stimuli were adjusted to make

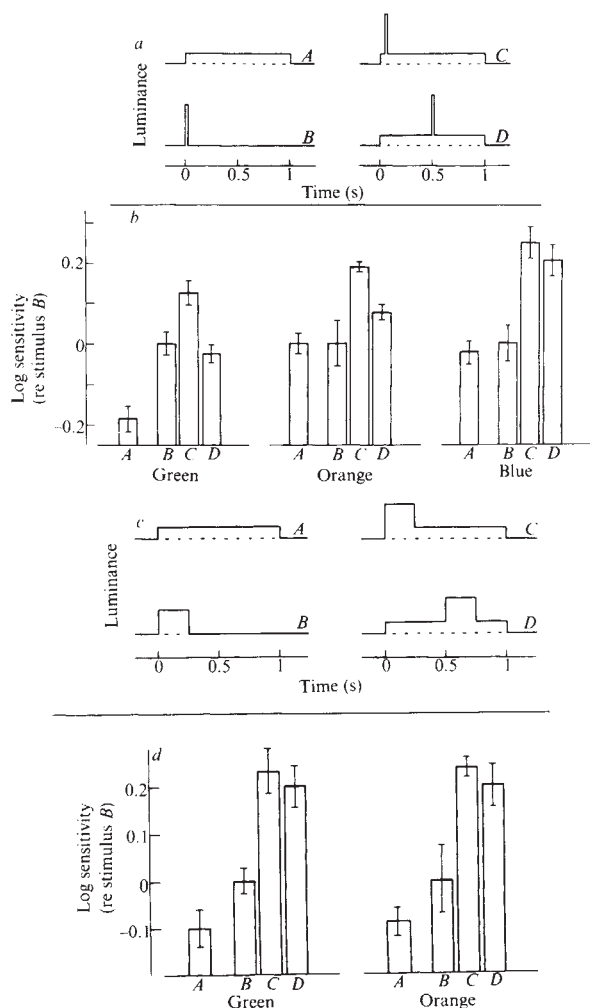


Fig. 1 Sensitivity to an 8-ms flash in the presence of a subthreshold flash lasting 1 s. *a*, Temporal waveforms of the four stimuli. Stimulus A is the conditioning flash alone; stimulus B is the test flash alone. In stimulus C, the test flash occurs 16 ms after the onset of the conditioning flash; in stimulus D, the test flash occurs with a delay of 500 ms. *b*, Sensitivities for the four temporal waveforms, for each of three colours. Sensitivity (mean) is expressed relative to that for stimulus B in each case. The vertical lines on each histogram block show ± 1 s.e.m. *c* and *d* are as for (a) and (b), respectively, except that the test stimuli lasted 250 ms as shown in the upper panel. Only green and orange stimuli were used.

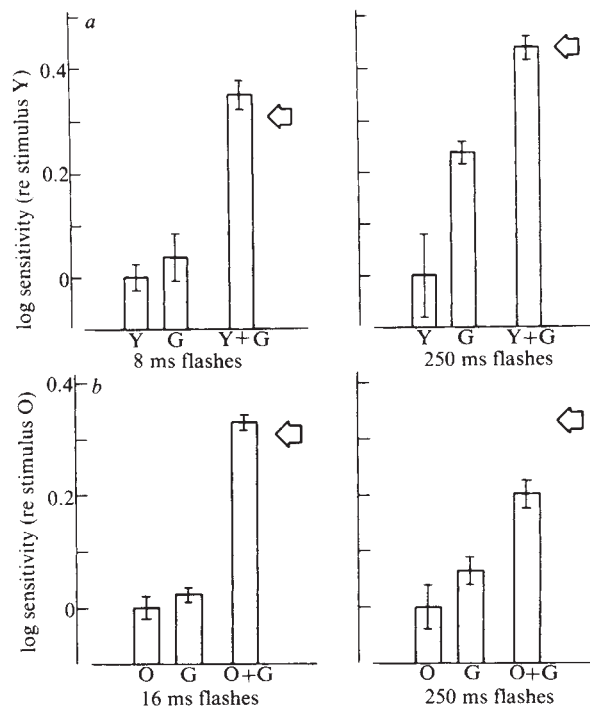


Fig. 2 Summation between stimuli with different wavelength composition. *a*, for yellow (Y) and green (G) stimuli. Summation was examined at two flash durations. The yellow and green flashes in each case were synchronous and of the same duration. Sensitivity is expressed relative to that for the yellow stimulus alone. The horizontal arrows show the sensitivity for the composite stimulus if there had been perfect summation of the sensitivities to the two components. *b*, for orange (O) and green (G) stimuli.

them more or less equally detectable alone. The sensitivity to the mixture was then determined. The arrows show the sensitivity that would have been achieved if there had been perfect summation. Both for short and for long flashes, summation was more or less perfect, implying that the two colours were detected by the same channels. This was found even though the long flashes appeared coloured. The green and yellow flashes were not easily distinguishable, however; both appeared to be green at threshold, suggesting that they were detected by the same colour-signalling channels. Figure 2b shows a similar experiment. The wavelengths of the two test stimuli were made more dissimilar (green and orange). Perfect summation was found only for the brief flashes, when both stimuli appeared achromatic. At long durations, the flashes appeared to be of different colours, and summation was incomplete. These results seem to be consistent with the observation that summation of the brightness of different wavelengths is almost perfect in flicker photometry⁷, whereas complex interactions are found when the stimuli are presented for 500 ms (ref. 8).

My results confirm King-Smith's suggestion that there are two sets of channel in the human visual system. One class signals the colour of a stimulus; this class is made up of several subsets, which each respond to a limited band of wavelengths. The second class of channel does not give information about the colour of a stimulus, but responds to a broad band of wavelengths. This paper has added the observation that the colour-coded channels give sustained responses to flashed stimuli, while the broad-band channels give transient responses at the onset of the flash. Single neurones in the monkey visual system show a similar difference: colour-opponent neurones give sustained responses, whereas broad-band non-opponent neurones respond transiently^{2, 5}.

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Solubilisation and partial purification of auxin-binding sites of corn membranes

THE demonstration by Hertel *et al.*¹ of reversible, high-affinity binding of auxins to membranous preparations from corn coleoptiles provided for the first time a reliable and readily accessible system for the study of potential receptor-phytohormone interactions. In a detailed investigation of the binding kinetics and specificity of this system, we presented evidence for the presence of two classes of auxin-binding sites with differing affinities and specificities^{2,3}. Site 1, with a K_D for the synthetic auxin 1-naphthalene-acetic acid (NAA) of 0.14 μM , was able to bind both auxins and physiologically inactive analogues, whereas site (K_D for NAA = 1.7 μM) displayed binding specificity compatible with that expected of an auxin receptor² and was located in a heavier membrane population, enriched in plasma membrane³. Auxin-binding activity can be readily solubilised from the membranes with Triton X-100 (ref. 2), but our attempts to purify such extracts further have met with only limited success. I report here that both binding sites can be conveniently removed from the membranes without detergent and purified approximately 100-fold in two steps.

Membranes were prepared from 40-80 g of corn coleoptiles (*Zea mays*, Kelvedon 33) by differential centrifugation between 4,000g and 80,000g, as before². The pellets were suspended using a Teflon glass homogeniser, in 0.1 volume (based on original tissue weight) of grinding buffer² and the suspension was added to 20 times its volume of Analaar acetone at 4 °C, with stirring. The clumped precipitate was separated from the yellow, opalescent

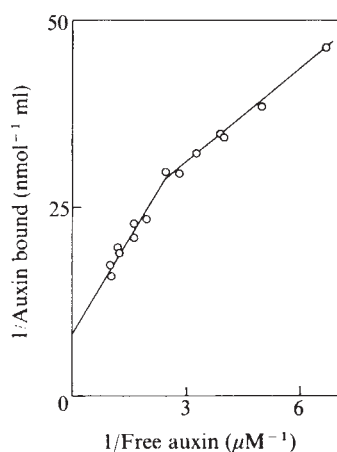


Fig. 1 Double reciprocal analysis of NAA binding to the crude membrane extract. Samples of 1 ml (each equivalent to 2 g of tissue) were dialysed against 1 ml of ^{14}C -NAA (61 mCi mmol⁻¹, Radiochemical Centre, Amersham) in binding buffer (0.25 M sucrose, 5 mM MgSO₄, 10 mM citrate-acetate, pH 5.5), supplemented with varying amounts of unlabelled NAA to give a range of equilibrium concentrations from 0.15 to 1.0 μM . Equilibrium required a minimum of 5-h dialysis at 4 °C using Dianorm cells (MSE, Crawley). Radioactivity in 750- μl aliquots from each half-cell was determined by liquid scintillation counting. The regression lines of the double reciprocal plot were fitted by the method of Wilkinson⁴; site binding constants were derived as described by Hunston⁵ for a two-site system. $K_1 = 0.17 \mu\text{M}$, $n_1 = 14.3 \text{ pmol g}^{-1}$; $K_2 = 1.4 \mu\text{M}$, $n_2 = 51 \text{ pmol g}^{-1}$.

supernatant by vacuum filtration or by low-speed centrifugation, washed with acetone and dried in a stream of nitrogen. The dried residue was extracted by homogenisation in DBB (a fivefold dilution of binding buffer, see Fig. 1 legend and ref. 2) and insoluble material was removed by centrifugation at 38,000g, 15 min.

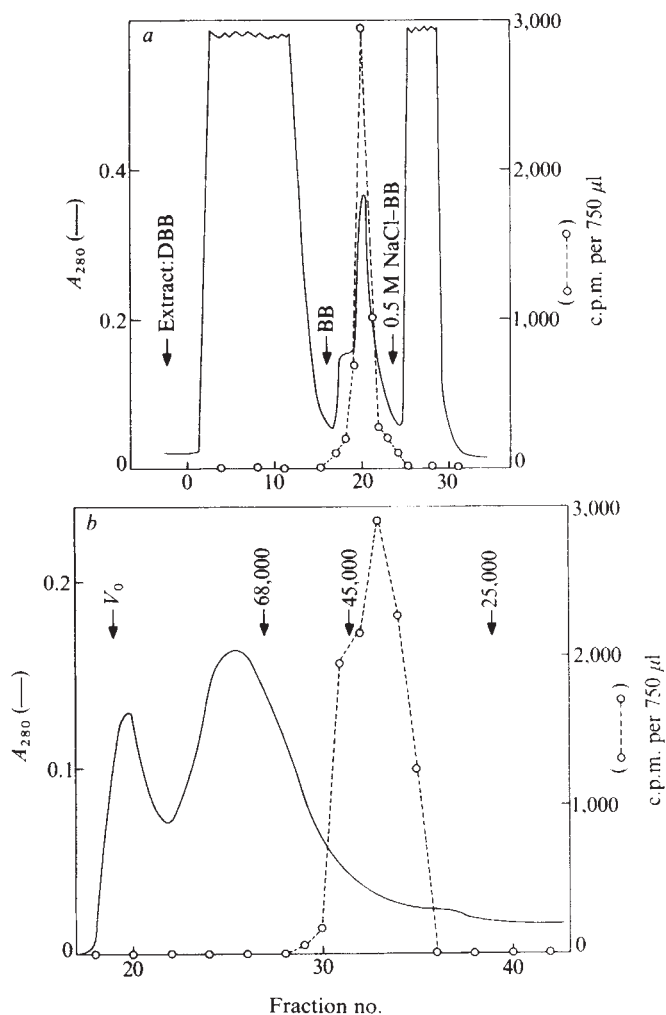


Fig. 2 Purification of auxin-binding proteins. The crude membrane extract was applied to a 5 × 1.5-cm column of DEAE Bio-Gel (Bio-Rad) equilibrated in DBB (fivefold diluted binding buffer; see legend to Fig. 1). The column was washed with DBB until A_{280} approached the baseline value. Elution was then continued step-wise with binding buffer (BB), followed by 0.5 M NaCl in binding buffer. Fractions of 2.5 ml were collected and aliquots (diluted to 1 ml with binding buffer) were assayed for auxin binding by equilibrium dialysis against 1 ml of 0.3 μM ^{14}C -NAA. Binding was determined from the radioactivity difference between each pair of half-cells. The fractions with binding activity were pooled, concentrated to 1.8 ml by centrifugal ultrafiltration (Amicon CF25 membrane cones) and applied to a column, 81 × 1.5 cm of Sephadex G100 (Pharmacia), equilibrated in binding buffer. Fractions of 2.0 ml were collected and assayed for auxin binding. *a*, DEAE Bio-Gel profile; *b*, Sephadex G100 profile. V_0 , void volume (ferritin). Arrows indicate elution peaks of standard proteins of the molecular weights indicated. Absorbance at 280 nm; —, ^{14}C -NAA binding.

Binding of ^{14}C -NAA to these crude membrane extracts was examined by equilibrium dialysis. Double reciprocal analysis (1/bound against 1/free) of the binding data yields biphasic plots (Fig. 1) similar to those obtained with intact membrane preparations², and suggesting that both sets of binding sites have been solubilised from the membranes. The dissociation constants ($K_1 = 0.17 \mu\text{M}$; $K_2 = 1.4 \mu\text{M}$) and the proportionality of binding sites ($n_1:n_2$) are in good agreement with the values for site 1 and

site 2 respectively deduced in earlier membrane experiments². The total yield of soluble binding sites in different experiments has ranged from 45 to 90% of that expected in the original membranes; the source of this variability is not clear.

When the buffer extracts were fractionated on DEAE Bio-Gel, auxin-binding activity was confined to the peak eluted with binding buffer (Fig. 2a). The auxin-binding material is heat labile (>90% loss of activity after 5 min at 90 °C), non-dialysable, and precipitates at 70% ammonium sulphate saturation. The post-DEAE binding fractions are positive to the Lowry reagent⁶ and nucleic acids were undetectable by organic micro-phosphorus analysis⁷. The binding species are therefore likely to be proteins. The pooled post-DEAE binding fraction was further purified on Sephadex G100, yielding a single, usually asymmetrical auxin-binding peak (Fig. 2b). The apparent molecular weight at the shoulder (fraction 31) is 47,300 and at the peak (fraction 33), 40,300. Overall purification in the two-column steps on the basis of c.p.m. bound per mg protein (mean of three experiments) is approximately 100-fold (crude extract = 1750; DEAE peak = 14,500; G100 peak = 171,800).

The asymmetric nature of the binding profile from Sephadex G100 could indicate a partial resolution of site 1 and site 2 proteins. Gel electrophoretic analysis of the post-Sephadex binding fractions indicates only limited heterogeneity, but additional purification and scaling-up are needed to assign the binding protein bands with greater confidence. Investigation of the effects of potential affinity labels on auxin binding to isolated corn membranes has permitted the tentative assignment of several of the amino acid residues in the binding site environment⁸. The availability of the solubilised binding proteins and the use of radioactive affinity labels will facilitate further characterisation of the binding sites.

The problem of establishing directly the significance of the binding sites in relation to the physiology of auxin action is more difficult to approach, but in principle it should be possible to incorporate the binding proteins in a synthetic membrane system, perhaps in conjunction with an ATPase, and look for a specifically auxin-mediated response such as proton pumping⁹.

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Relationship between amount of Epstein-Barr virus-determined nuclear antigen per cell and number of EBV-DNA copies per cell

EPSTEIN-BARR virus-determined nuclear antigen (EBNA) can be detected in all EBV-DNA-carrying cells^{1,2}. This includes Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) tumour biopsy cells and EBV-carrying lymphoid lines^{1–5}. Of the known EBV-associated antigens only EBNA is regularly associated with the presence of viral genetic material,

no matter whether the cell line produces virus or not^{6,7}. The composition and function of EBNA are largely unknown. Some recent reports suggest that EBNA is a protein that binds to DNA *in vitro*^{8,9}, and is associated with chromosomes *in vivo* at least in part¹. EBNA might be a virally determined or virally altered chromosomal protein of the non-histone type⁹. It is tempting to speculate that EBNA has a role in EBV-induced cell transformation ("immortalisation"¹⁰) and/or in the control of viral gene expression. There is a wide variation in the average number of EBV genome equivalents between different EBV-DNA-carrying cell lines. In virus non-producer cell lines this number is a better approximation of the true EBV genome number for individual cells than in virus producer lines, and is relatively stable over long periods of serial passage¹¹. The EBNA content of EBV-containing cell lines also seems to vary between different cell lines, as judged visually in the fluorescence microscope. We have measured the amount of EBNA per cell, by quantitative immunofluorimetry in different cell lines, in parallel with determining the average number of EBV-DNA copies, and established a correlation between these two parameters.

Eleven non-producer lines were tested. Three were derived from Burkitt's lymphoma (Raji¹², Namalwa¹³, Rael¹⁴), three were independently established somatic cell hybrids of Raji and Namalwa (RN2, RN17, RN21) (ref. 15), four were EBV-carrying lymphoblastoid cell lines of non-neoplastic origin (303L (ref. 16), 6410 (ref. 17), F265 (ref. 18), PESS (ref. 14)) and one was an EBV-converted derivative of the EBV-negative BL line Ramos (AW-Ramos (ref. 19)). The average number of EBV genome equivalents per cell was estimated by nucleic acid hybridisation as described previously². Briefly, 10-μg aliquots of cellular DNA, immobilised on nitrocellulose filters,

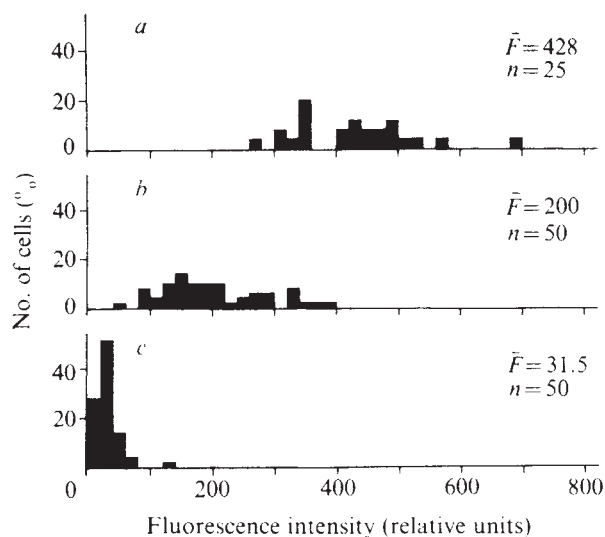


Fig. 1 Frequency distribution of EBNA fluorescence intensities of individual cells in cell lines that carry on average 160 (RN17, a), 60 (Raji, b) and 3 (Namalwa, c) EBV genome equivalents per cell.

were incubated for 96 h at 45 °C with 1 ng (1.5×10^5 c.p.m.) ³²P-labelled EBV complementary RNA (cRNA) in 0.3 ml 0.9 M NaCl-0.09 M trisodium citrate, containing 50% formamide. Negative (calf thymus DNA) and positive (NC37 (ref. 18) cellular DNA) controls were included in all experiments. The number of EBV genome equivalents per cell were calculated from the assumption that the molecular weight of EBV DNA is 10^5 (ref. 20), that of DNA from a diploid human cell 4×10^{12} , and that of DNA from a tetraploid cell 8×10^{12} .

For immunofluorimetry, small circles were drawn on a slide with a diamond. Cells of different cell lines were placed within different circles and diluted to allow a distance of 2–3 cell diameters between individual cells, in order to facilitate individual cell measurement without neighbour interference. Two

Raji cell samples were placed on each slide for reference. The cell patches were dried, fixed in acetone-methanol (2:1) for 3 min and incubated with an EBNA-positive serum from a BL patient (M.A., Kenya Cancer Council No. 1530, EBNA titre 1:160, VCA titre 1:640 and EA-R titre 1:40, diluted 1:10 with BSS) for 30 min. Thereafter the slides were incubated for 30 min with 1:10 diluted complement from a healthy human donor (I.E.) with no antibodies to EBNA or to any other EBV-determined antigens. Staining was carried out using fluorescein-labelled goat anti-human β_{1c} (Hyland laboratories, Los Angeles, California). Fluorescence emission of randomly selected individual cells was determined by microspectrofluorimetry as described previously²¹.

Figure 1 shows the frequency distribution of fluorescence intensity (FI) per cell in three lines. Raji and Namalwa were compared with one of their somatic hybrid derivatives, RN17. In each line fluorescence intensity per cell is distributed over a wide range. It is noteworthy that the FI values of the RN17 hybrid were not only higher than for either parent, but exceeded for most cells the additive value of the two parental lines.

EBNA fluorescence intensities of the same cell line (Raji) varied considerably between different experiments. To standardise the system, the mean value of the Raji cell patch on each slide was taken as reference value for the other lines measured on the same slide. This was chosen as a reference

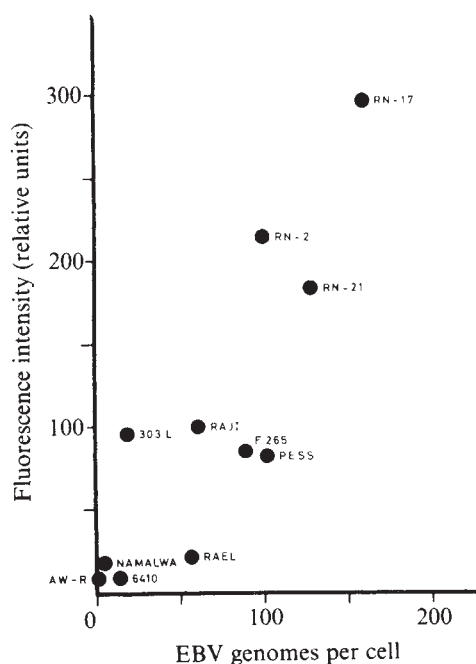


Fig. 2 Amount of EBNA per cell (mean cellular fluorescence intensity) plotted against average number of EBV genomes. Mean EBNA fluorescence value of each Raji standard cell population has been normalised to 100. All individual cell values of the other lines were computed in relation to the reference value. The corrected mean values were entered for each cell line. These mean values are based on the following number of cells measured: Raji 358, AW-Ramos 162, Namalwa 143, PESS 56, F265 15, 6410 40, Rael 25, 303L 13, RN-2 40, RN-17 40, and RN-21 40. The number of EBV genomes was estimated by filter nucleic acid hybridisation as described in the text.

because the variation between Raji patches on the same slide was much smaller than between different slides and different experiments. Following this procedure the EBNA content of the different cell lines could be compared and studied in relation to the number of EBV genome equivalents per cell. As shown in Fig. 2, there was a strong correlation between these two parameters ($r = 0.86$). Three lines, Namalwa, 6410 and AW-Ramos, had low numbers of EBV genome equivalents per cell and they also had the lowest quantity of EBNA per cell. Four lines, 303L, F265, PESS, and Raji, had intermediate EBNA and

EBV-DNA values. The three RN hybrids confirmed the impression gained from Fig. 1, in showing that the amount of EBNA per cell was not simply an addition of the parental values, but was amplified beyond the expected sum of the parents. Even more interesting was the fact that this amplification seemed to be directly proportional to the amplified EBV genome copy number, already demonstrated by Andersson¹¹. One cell line shows comparably low fluorescence intensity, Rael. It had a very low EBNA value per cell, but a somewhat higher EBV-DNA copy number than expected. In this context it is interesting to note that Brown *et al.*²² found that the binding of radiolabelled anti-EBNA antibodies to Raji nuclei was completely inhibited by nuclear extracts from several EBNA-positive cell lines, but only partially inhibited by Rael extracts. This suggests that Rael might lack some antigenic subcomponent of EBNA. This would also explain the low fluorescence intensity of Rael cells.

Obviously, the calculation of mean EBV-DNA and EBNA values masks the previously demonstrated cycle-dependent variation of both parameters²³ (also I.E. and D.K., unpublished). The multiple EBV genomes carried in non-producer Raji cells were shown to replicate during early S phase²³. We have found that EBNA increases continuously during the cell cycle, most of the increase occurring during the G1 phase (I.E. and D.K., unpublished). The fact that the mean values show a strong correlation suggests that physiological variations tend to 'even out' in large populations sampled on different occasions.

The relationship between mean EBV-DNA and EBNA values raises some intriguing questions. It is known that the 'immortalising', proliferative interaction of EBV with its host cell, the human or primate B lymphocyte, is entirely dependent on the restrictions of viral gene expression that prevent the entry of EBV-DNA-carrying cells into the invariably lethal viral cycle. Non-histone chromosomal proteins are believed to have a transcription-regulating role in differentiated cells^{24,25}. Since EBNA seems to be a virally induced or virally changed chromosomal protein, it is tempting to speculate that it may have an analogous role, perhaps in direct relation to the EBV cycle. A positive correlation might be expected between EBNA and EBV-DNA if EBNA regulates the transcription of the multiple viral genomes. Alternatively, or in addition, EBNA may have a role in transforming the normal B lymphocyte into an indefinitely proliferating, established cell line. As a third possibility EBNA might be simply a product of the latent viral genomes, without having any role in viral transformation or in the maintenance of viral latency. The correlation we have found might then be due to a viral gene-dosage effect.

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Proton pump coupled to cytochrome *c* oxidase in mitochondria

CYTOCHROME *c* oxidase is an important enzyme of aerobic metabolism, catalysing electron transport between cytochrome *c* and molecular oxygen. Apart from this essential redox reaction the enzyme must be involved in conservation of the released redox energy for utilisation in ATP synthesis at the so-called third site of oxidative phosphorylation. I report here that the redox activity of mitochondrial cytochrome *c* oxidase is coupled to the translocation of hydrogen ions across the inner mitochondrial membrane. This finding may be a clue to the role of cytochrome oxidase in energy conservation.

Cytochrome oxidase is located in the inner mitochondrial membrane, as are the other components of the respiratory chain

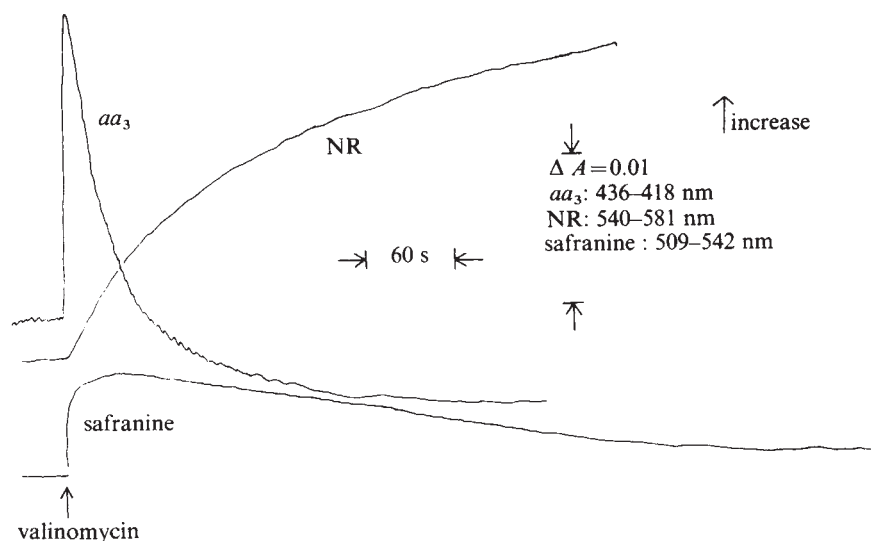
H^+ consumption gives rise to a pH gradient (ΔpH). The resulting electrochemical proton gradient (or protonmotive force, $\Delta\mu_H^+ = \Delta\psi - 60\Delta pH$) is suggested to be the driving force for oxidative phosphorylation^{2–4}.

The conformation of the haems in mitochondrial cytochrome oxidase changes in parallel to $\Delta\mu_H^+$, but does not correlate with either of its components, $\Delta\psi$ or ΔpH , alone⁵. Energisation of mitochondria, which is associated with the development of a protonmotive force of some 200 mV (positive outside)⁶, shifts the enzyme into a more low-spin conformation with associated changes in spectral, redox and kinetic properties^{5,7–11}.

The parallelism between the electrochemical proton gradient and the conformation of cytochrome oxidase is demonstrated in Fig. 1, where a diffusion potential of potassium ions is specifically induced across the mitochondrial membrane with the aid of the ionophore valinomycin. As demonstrated previously¹², the electrical potential difference can be monitored spectro-photometrically using safranine as a probe, while ΔpH can be recorded by means of the pH indicator neutral red¹³. As Fig. 1 shows, the electrical diffusion potential decays slowly, as expected, from a membrane system orders of magnitude less permeable to ionic species other than potassium. Neutral red shows progressively more acidity inside the mitochondria, with kinetics lagging behind the formation of the electrical potential. This suggests a relatively slow flux of protons into the mitochondria in response to the induced membrane potential. The spectral shift in ferric cytochrome oxidase (see refs 5,7–10) develops with kinetics similar to the safranine signal, but decays with much faster kinetics, similar to those of neutral red. Thus the conformation of cytochrome oxidase follows neither the $\Delta\psi$ nor the ΔpH component of the protonmotive force, but seems to change in parallel with their sum ($\Delta\mu_H^+$), decaying as the protonmotive force decays primarily due to increased internal acidity.

Cytochrome oxidase would be expected to be sensitive to the electrochemical proton gradient if the conformational change of

Fig. 1 Kinetics of membrane potential, pH gradient and spectral shift in ferric cytochrome *c* oxidase. The reaction mixture contained 0.2 M sucrose and 30 mM HEPES buffer, pH 7.2, with further additions of 10 μM rotenone, 0.33 mM EDTA, 0.9 mM sodium ferricyanide, oligomycin (9 $\mu g\ ml^{-1}$) and rat-liver mitochondria (3.6 mg protein per ml). In the experiment marked NR, a further 45 μM of neutral red was added, and in the experiment marked safranine, this dye was added to a concentration of 20 μM . Dual wavelength spectrophotometry was performed at the indicated wavelength couples, using cuvettes with 1.0-cm light path. Temperature was 23 °C. Each experiment was started after 30 s pre-incubation with the mitochondria, by addition of valinomycin (0.1 $\mu g\ ml^{-1}$). An upward deflection of the safranine trace indicates formation of an electrical membrane potential with positive polarity extramitochondrially¹². An upward deflection of the NR trace indicates acidification of the intramitochondrial space¹³ (at the high extramitochondrial buffering power used the dye measures intramitochondrial pH only¹³). Upward deflection of the trace marked aa_3 signifies the energy-linked spectral shift in the ferric cytochrome *c* oxidase^{5,8–10}.



and the ATP synthetase complex. Moreover, the enzyme seems to span the membrane, being exposed to some extent on both surfaces¹. Mitchell's chemiosmotic hypothesis of oxidative phosphorylation^{2–4} postulates that cytochrome oxidase catalyses transmembrane electron transfer from cytochrome *c* (on the outside) to oxygen, which is reduced on the inside of the membrane with consumption of H^+ ions. The vectorial electron transfer is suggested to give rise to a membrane potential ($\Delta\psi$) while the inside

the enzyme were coupled to the translocation of hydrogen ions across the membrane. Thus $\Delta\mu_H^+$ may drive protons into the mitochondria through a 'pump' coupled to the high- to low-spin transition in ferric haem. Conversely, and more important, redox activity of cytochrome oxidase may lead to the low-spin 'high energy' form of the enzyme, followed by transition to high-spin coupled to translocation of H^+ ions out across the membrane¹⁴.

This possibility was tested by pulsing aerobic mitochondria with

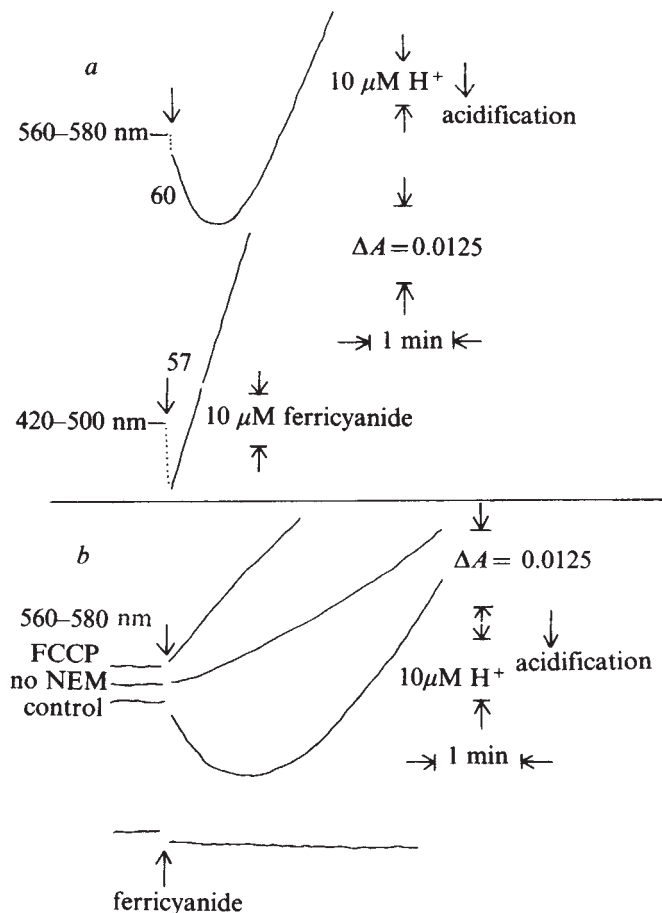


Fig. 2 Ejection of hydrogen ions coupled to oxidation of ferrocyanide in mitochondria. The reaction medium, 0.15 M KCl-1 mM HEPES, pH 7.4, was supplemented with $10\ \mu\text{M}$ rotenone, antimycin ($0.21\ \mu\text{g}\ \text{ml}^{-1}$), valinomycin ($0.67\ \mu\text{g}\ \text{ml}^{-1}$), oligomycin ($6.7\ \mu\text{g}\ \text{ml}^{-1}$), 0.67 mM EDTA, 0.33 mM NEM and rat-liver mitochondria²⁰ to a final concentration of 5.3 (a) or 6.0 (b) mg protein per ml. A dual wavelength spectrophotometer (Johnson Research Foundation Workshops, Philadelphia) was used at the wavelength couples indicated. An increase in the absorption difference results in an upward deflection of the trace. Light path was 1.0 cm and temperature was 23°C . Recording of extramitochondrial pH (a, upper trace, and b) was done in the presence of $10\ \mu\text{M}$ phenol red. The signal was calibrated with aliquots of HCl and found to be linear with (H^+) in the range measured. Ferricyanide production (a, lower trace) was recorded in the absence of the pH indicator. The signal was calibrated with known concentrations of ferricyanide. The reaction was started after 10 min preincubation by addition of 0.67 mM (a) or 0.33 mM (b, upper traces) ferrocyanide (at arrow). For the lower trace of (b), 0.67 mM ferricyanide was added as a control. Numbers adjacent to traces in (a) refer to initial rates in $\mu\text{M}\ \text{min}^{-1}$ of H^+ production (upper trace) and ferricyanide formation (lower trace) respectively. In (b) $0.8\ \mu\text{M}$ carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was present when indicated.

ferrocyanide as electron donor. The mitochondria were pretreated with rotenone and antimycin to minimise endogenous respiration, and oligomycin to abolish any proton influx linked to phosphorylation of endogenous ADP. The experiments were performed in a KCl medium with valinomycin to ensure that any electrogenic proton efflux would be balanced electrically by influx of potassium ions. *N*-ethyl-maleimide (NEM) was further added to inhibit the proton-phosphate symporter (or hydroxyl-phosphate antiporter) of the mitochondrial membrane¹⁵.

In these conditions oxidation of ferrocyanide by oxygen through cytochrome *c* and cytochrome oxidase indeed resulted in net release of protons into the extramitochondrial phase (Fig. 2a). The stoichiometry of the initial phase of the reaction was very closely one H^+ ion released per oxidised ferrocyanide, with ferrocyanide pulses between 0.13 and 1.35 mM. After the initial proton release there is alkalinisation of the mitochondrial suspension (Fig. 2) due to consumption of protons in the reduction of O_2 to water. In the presence of a proton-conducting uncoupling agent (FCCP, Fig. 2b), only the latter H^+ consumption is observed with the expected H^+/e^- stoichiometry of 1.0. Fig. 2b also shows that inhibition of the proton-phosphate symporter by NEM is a prerequisite for observation of net H^+ ejection. Leaving out either the valinomycin or the oligomycin retarded net H^+ release (not shown). The lack of these constituents in the ferrocyanide pulse experiments of Mitchell and Moyle¹⁶ may explain why they did not observe proton release. That the H^+ ejection is indeed coupled to oxidation of ferrocyanide and not to reduction of formed ferricyanide by endogenous substrates (though rotenone and antimycin were present to prevent this) was confirmed by the finding that ferricyanide itself produced negligible H^+ ejection (Fig. 2b). Moreover, inhibition of cytochrome oxidase by cyanide was fully inhibitory. Appropriate controls ensured that true pH changes were measured by the phenol red technique. Identical experiments using a pH electrode instead of the dye (not shown) confirmed that this was indeed the case.

It seems likely that the protons ejected into the extramitochondrial space originate in the mitochondrial matrix rather than in membrane protein components, for with large ferrocyanide pulses the number of protons ejected exceeds the content of respiratory chain complexes by a factor larger than 25. Moreover, with neutral red as an indicator of intramitochondrial pH (ref. 13) a ferrocyanide pulse caused rapid alkalinisation (Fig. 3), which was highly inhibited by uncoupling agents, and the extent of which was enhanced by NEM.

These results suggest that the oxidation of ferrocyanide by oxygen as catalysed by mitochondrial cytochrome *c* oxidase is coupled to the activity of an electrogenic proton pump. The H^+ translocation is apparently linked to a conformational change in cytochrome *aa*₃ with an associated spin state transition in the haem structure. The basic mechanism thus resembles the Bohr effect in haemoglobin¹⁷ though in cytochrome oxidase the protons are translocated across a membrane with conservation of energy. The

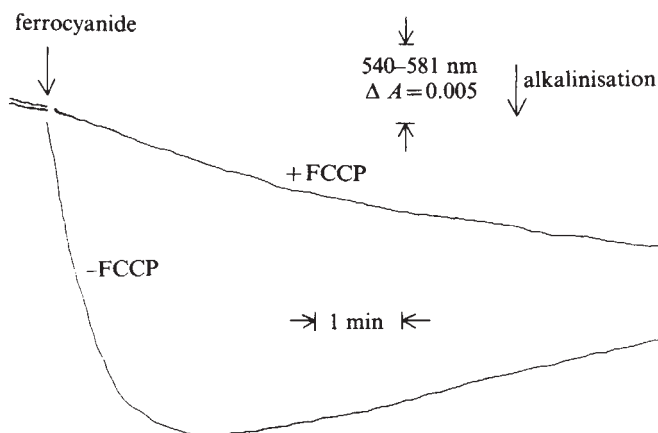


Fig. 3 Intramitochondrial alkalinisation coupled to ferrocyanide oxidation by mitochondria. Reaction mixture: 0.12 M KCl-30 mM HEPES-Tris buffer, pH 7.4 with additions as listed in the legend to Fig. 2. Rat-liver mitochondria $5.2\ \text{mg}\ \text{protein}\ \text{ml}^{-1}$. Neutral Red $67\ \mu\text{M}$. The experiment was performed as described in Fig. 1, by addition of 0.67 mM ferrocyanide in the absence (-FCCP) or presence (+FCCP) of $0.8\ \mu\text{M}$ FCCP.

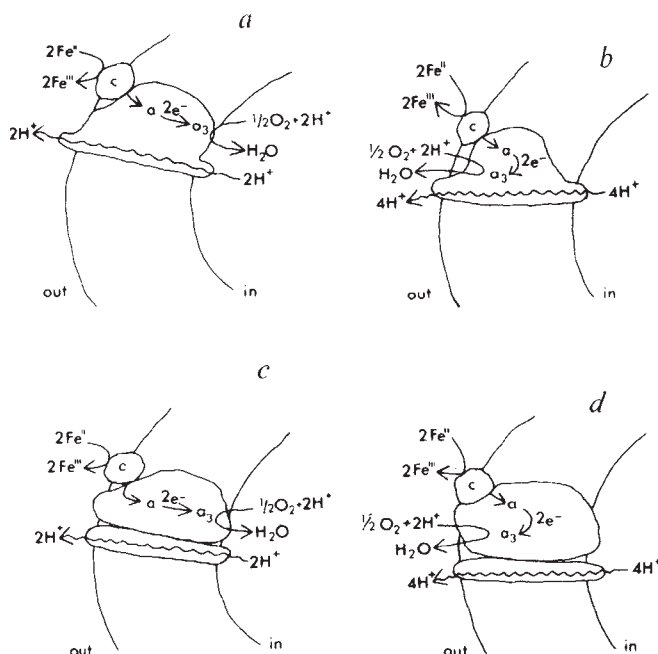


Fig. 4 Possible arrangements of the proton pump coupled to mitochondrial cytochrome c oxidase. Symbols: *c*, *a* and *a₃* indicate the respective cytochromes; Fe^{II} and Fe^{III} indicate ferro- and ferricyanide respectively. For further details, see text.

observed H^+/e^- stoichiometry of 1 means that transfer of two electrons to $1/2 \text{O}_2$ leads to separation of four charges across the mitochondrial membrane and not two as envisaged in the chemiosmotic hypothesis¹⁸. As shown in Fig. 4, however, there are at least four possible arrangements of cytochrome oxidase and the proton pump in the mitochondrial membrane. Alternatives *a* and *c* retain the transmembrane electron transfer feature of cytochrome oxidase⁴ but add a proton pump with a stoichiometry of $1 \text{H}^+/\text{e}^-$ either as part of (the subunit structure of) the enzyme itself (*a*), or as a separate molecular entity (*c*). If electron transfer is not electrogenic (*b* and *d*), the proton pump must be responsible for the entire charge separation and should thus operate with a stoichiometry of $2\text{H}^+/\text{e}^-$ (or $4\text{H}^+/2\text{e}^-$) to fit the present data. Distinction between alternatives *a* and *b* compared with *c* and *d* should be possible by studying H^+ translocation in liposomes incorporated with purified cytochrome oxidase¹⁹. Studies of this kind have shown (my and H. T. Saari's unpublished work) that the proton pump described is an intrinsic property of this enzyme (*a* and *b*, Fig. 4) rather than a separate molecular entity (*c* and *d*, Fig. 4). A distinction between alternatives *a* and *c* compared with *b* and *d* may, however, be more difficult experimentally.

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Non-allelic variants of histones 2a, 2b and 3 in mammals

THE genome of eukaryotes is organised in nucleoprotein fibres formed by the interaction of the DNA with small basic proteins, the histones, and by specific interactions between certain histones¹. To gain more insight into the molecular organisation of chromosomes it is necessary to determine in detail the complexity and variability of the histones. The chromosomes of eukaryotes contain five major types of histones. One of these, the very lysine-rich H1, consists of a small number of polypeptides which differ slightly in primary structure and which are present in different relative amounts in different tissues². The other four histones, which are involved in histone-histone interactions, were considered homogeneous and invariable until it became possible to resolve H2a, H2b and H3 into variants which exhibit tissue-specific variation by polyacrylamide gel electrophoresis in presence of non-ionic detergents³ (Fig. 1). We report here on the primary structure of the variants of mammalian histones 2a, 2b and 3.

Individual histone components were isolated from calf thymus and several other mammalian tissues by preparative polyacrylamide gel electrophoresis (in the presence of Triton X-100) of fractions enriched in individual histone classes by chemical fractionation⁴ or by ion exchange chromatography on CM-cellulose (S.G.F. and A.Z. in preparation). Direct comparison of the amino acid compositions of the electrophoretically pure variants of the different histone classes indicated the presence of one to three amino acid substitutions in each case⁵. To determine the molecular differences between the variants in more detail, we analysed their tryptic or thermolytic peptides resolved by microfingerprinting on microcrystalline cellulose plates.

After visualisation by spraying with 10% triethylamine and 0.01% fluorescamine in acetone^{6,7}, the peptides (or in some cases only non-identical peptides) were eluted from the plates, hydrolysed and subjected to amino acid analysis. The position within the protein of the eluted peptides was determined by homology in amino acid composition with the published amino acid sequences for the major components of these histones in calf thymus^{8–10}. With the exception of the core of H3.3, no ambiguities were encountered in localising substitution sites by composition differences. The substitutions determined by these methods agree with the differences in composition found by amino acid analysis of the intact proteins.

The tryptic peptide maps of calf H2a.1 and H2a.2 were identical with the exception of one additional spot seen in H2a.2. This spot was found to be Ser-Arg (actual analysis: serine, 1.94; arginine, 1.06) arising from a serine-threonine substitution at position 16, known also to occur in the rat¹¹. All other tryptic peptides from H2a.2 were homologous to H2a.1 peptides. Thermolytic digestion of the insoluble tryptic core of H2a.2 produced a peptide homologous with residues 43–51 (proline, 1.16; glycine, 2.25; alanine, 2.11; valine, 2.01; methionine, 0.53; tyrosine, 0.92) except that a methionine residue was present and leucine 51 was absent. Cleavage of H2a.2 with cyanogen bromide, isolation of the fragments by preparative polyacrylamide gel electrophoresis and amino acid analysis

confirmed this methionine-leucine substitution at position 51 and the serine-threonine substitution at position 16.

Both tryptic and thermolytic peptide maps of mouse ascites H2b.1 and H2b.2 were identical. Since differences in hydrophobicity determine the resolution of histones in non-ionic detergent electrophoresis, we examined the hydrophobic regions. Peptide T12 (ref. 12) was homologous with residues 73-79 except that in H2b.2 it contained an additional serine

except that it contained no cysteine and had one serine (serine, 0.92; glutamic acid, 3.06; alanine, 2.11; leucine, 0.93; tyrosine, 0.97). Thus a serine-cysteine substitution exists at position 96, in agreement with the findings of Patthy and Smith¹³ and known to occur also in shark, carp and chicken¹⁴⁻¹⁶. From thermolytic digestion of the tryptic cores of calf and sheep H3.3 we have confirmed that it also contains the above serine-cysteine substitution. The remainder of the core peptides are homologous with the sequence of H3.1 except that we cannot find a peptide corresponding to residues 88-90 (Ala-Val-Met). Instead we recover a peptide with the composition: alanine, 1.03; isoleucine, 0.90; glycine, 1.07. The most likely substitution pattern indicated by our data would be: isoleucine instead of valine 89 and glycine instead of methionine 90. The latter substitution is not conservative and occurs rarely¹⁸. Pea H3, however, also contains a 'helix-breaker' (serine) at position 90 (ref. 17). The arrangement of amino acids in this region of H3.3 has to be confirmed by direct sequence studies.

Figure 2 summarises our findings for all the mammalian H2a, H2b and H3 variants which we have characterised so far. All the substitutions (except for position 16 in H2a) are located in hydrophobic regions where the 'helix probability'¹⁹⁻²⁰ is

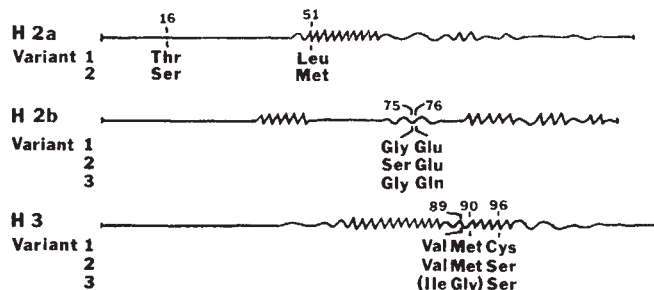
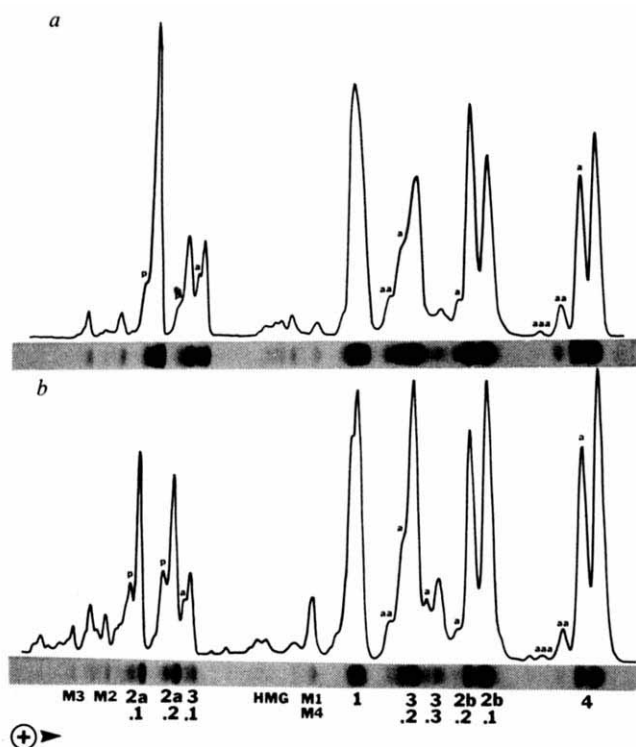


Fig. 2 Substitution sites in the mammalian histone variants. The degree of high helix probability²⁰ in different regions of the proteins are symbolically indicated by wavy lines.

liver (b) as resolved by electrophoresis (from left to right) on 12% polyacrylamide gels containing 7.5 M urea, 6 mM Triton X-100 and 5% acetic acid. The proteins were stained with amido black and the gel scanned at 300 nm. 1, 2a, 2b, 3, and 4 = major histone species; .1, .2, .3 = variants of a given histone species; M1, M2, M3, M4 = minor histone species with properties characteristic for histones but amino acid compositions not closely related to any of the five major histone species²³; HMG = 'high mobility group' proteins²⁷; a = acetylated, p = phosphorylated forms. Note the difference in the relative amounts of H2a.1 against H2a.2, H2b.1 against H2b.2, H3.1 against H3.2 and H3.3 in the two tissues.

residue and no glycine (serine, 1.79; glutamic acid, 1.39; alanine, 2.18; isoleucine, 1.05; arginine, 0.59). This indicates a serine-glycine substitution at position 75. Calf thymus and other mammalian tissues contain a minor component which migrates similarly to H2b.2 on electrophoresis. The tryptic peptide map of this minor component from calf thymus is identical to those of mouse and calf H2b.1 except that it possesses one extra spot. The composition of this spot (serine, 1.23; glutamic acid, 1.19; glycine, 0.93; alanine, 1.83; isoleucine, 0.85; arginine, 0.96) is identical with T12 (which is also present on the map) of H2b.1 yet it moves considerably farther in the electrophoretic and chromatographic dimensions. The most likely explanation for this is the substitution of a glutamine for a glutamic acid at position 76 in a new H2b variant, H2b.3. This is consistent with its amino acid composition being identical to H2b.1. The simultaneous presence of peptide T12 in two spots is probably due to partial deamidation of glutamine 76.

Tryptic maps of calf H3.1 and H3.2 are identical but the thermolytic map of H3.2 shows a major spot in a region where H3.1 shows a cluster of faint spots. Analysis of the H3.2 spot revealed a peptide homologous with residues 92-99 of H3.1

sensitive to small changes in amino acid side chains. All but one of the substitutions are conservative and result in small changes in the detergent affinity except for the cysteine-serine substitution in H3 which has a large effect on detergent affinity. This is consistent with the observed general correlation between detergent affinity and helix probability^{2,21}, since, although cysteine and serine are similar in configuration, they belong to different categories of helix promoting ability¹⁹. Our findings for the histone variants demonstrate the extreme sensitivity of our method to small differences in hydrophobic properties of proteins, which facilitates the separation of proteins differing in a single conservative amino acid substitution involving neutral amino acid residues.

Except for H2b.2, which has only been found in the mouse²², all variants have been found simultaneously in all tissues of all mammals examined, although in different relative amounts²³. The simultaneous presence of all variants in plasmacytomas and tissue culture lines derived from inbred mice, indicates that the genes for the different histones are non-allelic. Furthermore, all variants are synthesised simultaneously in HeLa cells²⁴ from mRNAs with slightly different properties²¹. In the sea urchin, different H2a and H2b variants are synthesised at different stages of embryonic development which suggests the existence of different histone operons^{25,26}.

These observations, together with the parallel conservation of the non-allelic histone variants throughout the evolution of at least the mammals, suggest that the histone variants have independent essential functions. The histone classes H2a, H2b, H3 and H4 interact by non-ionic forces to form highly ordered heteropolymers which constitute the central structural element of eukaryotic chromosomes²⁸. The amino acid substitutions distinguishing the histone variants are located in the

hydrophobic regions which are involved in these histone-histone interactions. It is therefore conceivable that chromosomal fibres containing histone complexes composed of different histone variants differ significantly in their physical properties and that different histone variant populations are necessary for optimal function of specific regions of the genome in nuclei of cells with different metabolic patterns.

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Formation of amino acids from elemental carbon by contact glow discharge electrolysis

THE formation of amino acids in various inferred prebiotic conditions has been widely studied^{1,2}. Recently we reported syntheses of amino acids from aliphatic carboxylic acids and ammonia³⁻⁴, and from aliphatic amines and formic acid⁴⁻⁵ by means of contact glow discharge electrolysis (CGDE)⁶. We describe here the formation of amino acids and urea from elemental carbon in the presence of aqueous ammonia by CGDE. By this reaction and the following process, the elemental carbon of the electrode was converted into the bio-organic compounds.

The reaction tube used for CGDE was as illustrated in Fig. 1. The carbon rod (Irikawa, IBGC-55; diameter 5 mm; specific

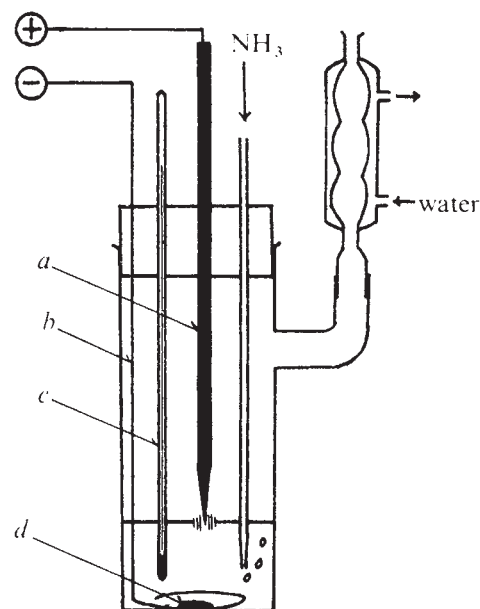


Fig. 1 Reaction tube for CGDE. *a*, carbon anode; *b*, platinum cathode; *c*, thermometer; *d*, magnetic stirrer bar.

gravity 1.65; electrical resistance 250 Ω) was used as the anode and platinum wire was used as the cathode. All glassware used was heated for 1 h at 500 °C before CGDE to eliminate possible contamination of amino acids⁷. Anhydrous ammonia was bubbled into the twice-distilled water (about 10 ml) in the reaction tube and then CGDE was performed for 35 min. The applied electric current was 60 mA at 600-1200 V. CGDE was conducted in conditions of ammonia gas saturation and the reaction temperature was kept at about 30 °C by cooling the reaction tube in a methanol-dry ice bath. After the reaction was over, the solution was heated at 90 °C for 23 h in a sealed tube and was evaporated to almost dryness under reduced pressure. The residue was hydrolysed with 2 ml of distilled 6 N hydrochloric acid in a sealed tube under reduced pressure. After heating at 120-130 °C for 23 h, the sealed tube was opened and the mixture evaporated to dryness under reduced pressure. The residue in the tube was diluted appropriately and was analysed by an amino acid analyser (Yanagimoto LC-5S).

A typical result of the amino acid analysis is shown in Fig. 2B. Several control experiments were carried out without applying CGDE, and only trace amounts of glycine were detected (Fig. 2A). The weight loss of the carbon anode after the CGDE reaction was 5.6 mg. Thus the conversion of elemental carbon to organic carbon was at least 0.2% (during CGDE a very small amount of carbon

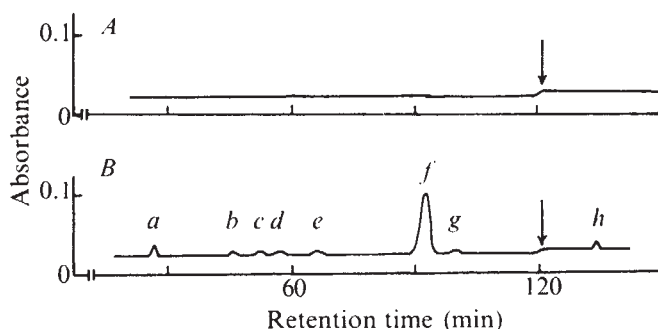


Fig. 2 Amino acid analysis. *A* Control experiment without CGDE. A trace amount of glycine ($\sim 10^{-10}$ mol) was detected. The peak is not visible in the chart. *B*, Reaction conditions were: electric current, 60 mA; reaction time 35 min. Compounds found in the reaction mixture were *a*, urea (6.1×10^{-7} mol); *b*, aspartic acid (1×10^{-9} mol); *c*, threonine (1×10^{-9} mol); *d*, serine (8×10^{-10} mol); *e*, glutamic acid (5×10^{-10} mol); *f*, glycine (4.5×10^{-8} mol); *g*, alanine (1×10^{-9} mol); *h*, unknown peak. Arrows indicate buffer change.

powder was found in the reaction mixture, so 0.2% is the minimum).

To our knowledge, this reaction is the first example in which amino acids and urea were obtained directly from elemental carbon. These facts suggest a new possibility for the prebiotic synthesis of amino acids in prebiotic conditions on the primordial Earth.

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Enzyme hydration may explain catalytic efficiency differences among lactate dehydrogenase homologues

HOMOLOGOUS enzymes of species with different cell temperatures usually display temperature-adaptive differences in their catalytic properties¹⁻⁷. Thus enzymes of low cell temperature species characteristically exhibit higher specific activities and lower activation free energies and enthalpies than the homologous enzymes of high cell temperature species²⁻⁷. For lactate dehydrogenase (LDH, E.C.1.1.1.27; lactate: NAD⁺ oxidoreductase), a single M₄ (muscle type) LDH molecule from a cold-water fish can catalyse the conversion of pyruvate to lactate three times as rapidly at 5 °C as an M₄-LDH from a mammal⁸. In this paper we present evidence that these temperature-compensating differences in catalytic efficiency may derive in part from different amounts of exergonic hydration of the enzymes during the activation step in catalysis. Lowering the activation free energy ($\Delta G^\ddagger$) 'barriers' to enzymic reactions by way of the reversible hydration of amino acid side chains and/or polar peptide linkages permits the adjustment of catalytic efficiencies without necessitating the modification of amino acid sequences in the active sites of the enzymes.

Attempts to explain mechanistically the differences in catalytic efficiency which exist among homologous forms of the same enzyme must face the following paradox: even though different homologues vary significantly in catalytic efficiency, the amino acid sequences of their active (catalytic) sites are virtually identical⁸⁻¹⁰. Where amino acid substitutions in active sites have occurred, they tend to be extremely conservative⁸. It therefore seemed reasonable to consider non-active site regions of the enzyme when attempting to explain the differences in catalytic efficiency among differently temperature-adapted LDH homologues.

Recently a rate-enhancement mechanism which is not based on active site conformations has been proposed, the 'protein group transfer-hydration mechanism'¹¹. Rate enhancement by this mechanism results from the exergonic transfer of protein groups (amino acid side chains and peptide backbone linkages) between the hydrophobic interior of the enzyme and the enzyme-water interface during the activation step in catalysis. These exergonic transfers will lower the free energy of the activated complex, thereby enhancing its probability of formation and accelerating the reaction rate. For example, the transfer of a peptide linkage from the interior of the enzyme, where it is relatively or fully dehydrated, to the enzyme-water interface, where a strongly exergonic hydration of the group will occur, can lower $\Delta G^\ddagger$ by as much as 1,100 cal mol⁻¹

(ref. 11). Since changes in $\Delta G^\ddagger$ of only 100-200 cal mol⁻¹ are adequate to approximately double the rate of a reaction², even slight changes in the exposure (hydration) of protein groups could be sufficient to significantly modify the catalytic efficiency of an enzyme. Since conformational changes seem to have a generally important role in enzymic catalysis¹², numerous changes in protein group exposure to water will occur during the activation event. Thus the protein group transfer-hydration mechanism of rate enhancement seems a generally accessible mechanism for rate enhancement of all types of enzymes.

In the present study we have tried to determine if different amounts of exergonic hydration processes during the activation step occur in the LDHs of warm- and cold-blooded vertebrates. Three experimental tests, all of which are based on the energy and volume changes which accompany protein group transfer-hydration processes, were carried out.

First, the transfer of all types of protein groups from a relatively non-polar environment, such as the enzyme interior, to water occurs with a volume decrease arising from the dense organisation of water around the hydrated protein group¹³. Thus a rough estimate of the involvement of protein group transfer-hydration processes in catalysis can be obtained from the volume change which occurs during the activation event. The activation volume ($\Delta V^\ddagger$) equals the difference in system (enzyme plus solvent) volume between the ground-state enzyme-substrate complex and the activated complex¹⁴. The activation volume of a reaction can be determined by estimating the effect of hydrostatic pressure on the maximal velocity ($V_{\max}$) of the reaction¹⁴. If the highly efficient LDHs of low cell temperature organisms become more hydrated during the activation step

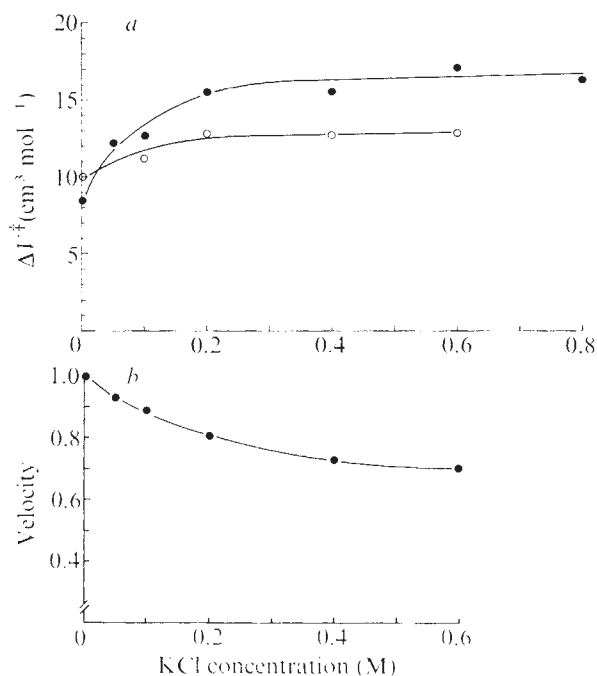


Fig. 1 *a*, Effect of KCl concentration on $\Delta V^\ddagger$ of the halibut (●) and turkey LDH reactions (○). *b*, Effect of KCl concentration on $V_{\max}$ of the halibut LDH reaction. All assays were carried out using fully purified LDHs (Dr Nathan O. Kaplan). The assay medium contained, in a total volume of 5.0 ml, 80 mM Tris-HCl buffer (pH 7.5 at the assay temperature, 10 °C), 0.025 mM NADH, varying concentrations of pyruvate and KCl, and enzyme, which was added last to initiate the reaction. The change in A_{340} was followed with time. The high pressure assay system was as described by Low and Somero^{11,15}. The inhibition data were obtained from assays run at 1 atm. Theoretical $V_{\max}$ values were determined using several pyruvate concentrations; data were analysed using plots of $1/\text{velocity}$ against $1/\text{pyruvate concentration}$. For determining activation volumes the reactions were run at 1,000 and 5,000 pounds per square inch (68 and 340 atm, respectively). The logarithm of $V_{\max}$ for the different LDHs varied linearly with pressure over the pressure range of 1 to approximately 500 atm.

than the LDHs of birds and mammals, then a larger negative contribution to $\Delta V^\ddagger$ will be found for the cold-adapted enzymes.

A second experimental test of this hypothesis is provided by the fact that the free energy changes which accompany these transfer processes are strongly influenced by the salt composition and concentration of the medium¹⁵. Salt conditions which make these transfers less exergonic would be expected to have two measurable effects. First, the velocity of the reaction will be reduced since the transfer will be energetically less favourable. Second, by impeding the hydration of transferred protein groups, salts will increase $\Delta V^\ddagger$. Thus a comparative estimate of the parts played by protein group transfer-hydration processes can be obtained by measuring salt effects on $V_{\max}$ and $\Delta V^\ddagger$. If these transfer processes are responsible for the higher specific activity of LDHs of low cell temperature species, then the salt sensitivities of $V_{\max}$ and $\Delta V^\ddagger$ should be greater for the cold-adapted enzymes.

A third approach to analysing the parts played by these transfer-hydration processes in LDH homologues is based on the fact that these transfers occur with substantial enthalpy changes^{16,17}. Also, as in the case of transfer free energies and volumes, transfer enthalpies will be affected by solute conditions¹⁸. Thus, if protein group transfers contribute also to the low $\Delta H^\ddagger$ values of enzymes of cold-adapted organisms, then the $\Delta H^\ddagger$ values of cold-adapted LDHs should be more salt-sensitive than the values of avian and mammalian LDHs.

The data presented in Figs 1 and 2 and in Table 1 support all of the predictions made above. Figure 1 illustrates the manner in which a weakly salting-out salt, KCl, affects the $\Delta V^\ddagger$ and $V_{\max}$ of the LDH reactions. Increasing KCl concentrations titrate both the rate and $\Delta V^\ddagger$, with essentially complete titration occurring at KCl concentrations of approximately 0.2–0.4 M. The $V_{\max}$ and $\Delta V^\ddagger$ values of the halibut reaction are significantly more salt-sensitive than the values for the turkey LDH reaction (Fig. 1, Table 1), the result predicted on the basis of a greater degree of exergonic exposure and hydration of protein groups during the activation step in the halibut LDH reaction.

Table 1 lists the effects of 0.6 M KCl on $V_{\max}$ and $\Delta V^\ddagger$ for LDH reactions of several warm- and cold-blooded species. The highly efficient teleost fish LDHs are significantly more inhibited than the other LDHs, and the salt-induced increase in $\Delta V^\ddagger$ is also largest for the teleost enzymes. The shark LDH is intermediate in salt sensitivity between the teleost and avian-mammalian enzymes. Elasmobranch LDHs are also intermediate between teleost and avian-mammalian LDHs in catalytic efficiency¹⁹.

Figure 2 illustrates the effects of 0.6 M KCl on the $\Delta H^\ddagger$ values of the halibut and turkey LDH reactions. KCl has virtually no effect on the $\Delta H^\ddagger$ of the turkey LDH reaction. $\Delta H^\ddagger$ values of 10,600 and 10,200 cal mol⁻¹ were observed in the absence and presence, respectively, of KCl. For the halibut reaction, addition of 0.6 M KCl to the assay medium increased $\Delta H^\ddagger$ from 7,520 cal mol⁻¹ to 9,260 cal

mol⁻¹. Only approximately 2 cal mol⁻¹ of this increase are due to changes in pressure-volume work ($\Delta H^\ddagger = \Delta E^\ddagger + P\Delta V^\ddagger$) arising from the salt-induced increase in $\Delta V^\ddagger$ (Table 1, Fig. 1). The Arrhenius plot for the halibut LDH reaction in the presence of 0.6 M KCl displays a 'break' near 25 °C. This discontinuity in slope may be the result of a salt-induced change in enzyme structure at higher temperatures.

The finding that the size of $\Delta V^\ddagger$ at 0.6 M KCl is greatest for the teleost LDHs (Table 1) may be of importance in understanding the structural differences which exist among the LDHs. The $\Delta V^\ddagger$ observed at KCl concentrations between approximately 0.2 and 1.0 M may be a rough measure of the change in protein void volume which arises from alterations in the packing efficiency of amino acid residues during the activation event¹³. That is, this KCl-insensitive component of $\Delta V^\ddagger$ is a 'structural' activation volume as distinct from the 'hydration density' component of $\Delta V^\ddagger$, which is due to water organisation around transferred protein groups and which is titrated by low KCl concentrations¹³. Within this conceptual framework the larger $\Delta V^\ddagger$ at high salt concentration found for the teleost

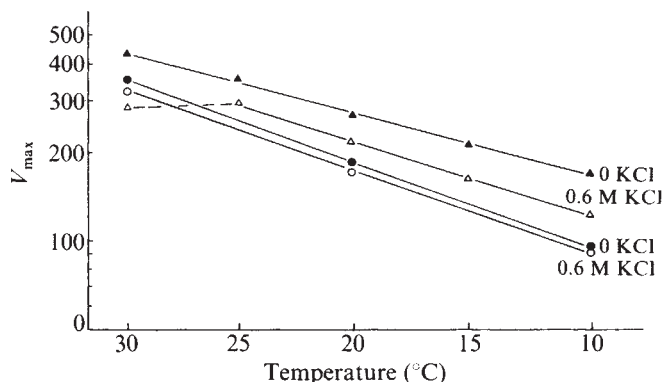


Fig. 2 Arrhenius plots showing the effect of 0.6 M KCl on the activation enthalpy value of the halibut (triangles) and turkey LDH reactions (circles). Assay conditions were as given in Fig. 1. Theoretical $V_{\max}$ values were determined using several concentrations of pyruvate. $\blacktriangle$, zero KCl; $\triangle$, 0.6 M KCl; $\bullet$, zero KCl; $\circ$, 0.6 M KCl.

LDHs is interpreted as being due to a greater degree of enzyme unfolding during catalysis. This increased expansion of enzyme structure during catalysis by the teleost LDHs may be possible because of the smaller amounts of secondary structure in these enzymes relative to the LDHs of birds, mammals and elasmobranchs¹⁹. Thus, the more flexible LDHs of cold-adapted species may be capable of more fully unfolding their conformations to expose protein groups during catalysis. This interpretation is consistent with the finding that the thermal stabilities of enzymes are frequently inversely proportional to their catalytic efficiencies^{3,4}.

In summary, the apparent necessity for maintaining the same amino acid sequences in both the binding and catalytic loop

Table 1 Effects of 0.6 M KCl on $\Delta V^\ddagger$ and $V_{\max}$ for LDHs from warm- and cold-blooded organisms

Species	$\Delta V^\ddagger$, no KCl*	$\Delta V^\ddagger$, 0.6 M KCl*	600-0*	% Inhibition
Rabbit	9.9 ± 0.7 (3)	12.3 ± 0.5 (3)	2.4	11
Turkey	10.0 ± 1.4 (6)	12.9 ± 1.4 (6)	2.9	5
Shark (<i>Carcharodon carcharias</i>)	7.8 ± 1.3 (7)	14.2 ± 1.3 (6)	6.4	9
Tuna (<i>Thunnus thynnus</i>)	9.4 ± 1.5 (9)	19.6 ± 0.8 (9)	10.2	17
Halibut (<i>Hippoglossus stenolepis</i>)	8.5 ± 0.9 (4)	16.0 ± 1.4 (11)	7.5	29
Haddock (<i>Melanogrammus aeglefinus</i>)	8.4 ± 1.4 (5)	17.2 ± 2.3 (3)	8.8	20

*Unit: cm³ mol⁻¹. Mean ± 1 s.d. (sample size) for columns headed no KCl and 0.6 M KCl.

†Inhibition of $V_{\max}$ due to addition of 0.6 M KCl to the assay medium. Theoretical $V_{\max}$ values were determined using several pyruvate concentrations; data were analysed using plots of 1/velocity against 1/pyruvate concentration. Theoretical $V_{\max}$ values were used because KCl inhibited the reaction competitively as well as non-competitively.

regions of all homologues of M₄-LDH (ref. 8) does not hinder the functional adaptation of each LDH to the temperature conditions it experiences. For the LDHs of low cell temperature species, which must function at low and, in many cases, highly variable temperatures, adaptive reductions in $\Delta G^\ddagger$ and $\Delta H^\ddagger$ seem to have been achieved by means of increased exergonic exposures of hydrophilic protein groups during the activation step in catalysis. Thus, a higher catalytic efficiency (lower $\Delta G^\ddagger$) and a reduced temperature dependency (lower $\Delta H^\ddagger$) can both be achieved by evolutionary changes in enzyme structure at regions remote from the active site.

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Calculated forbidden band gap in periodic protein models indicating them to be insulators

THE idea of electron and hole transport between distant sites in biological macromolecules, especially in fibrous proteins¹ prompted quantum chemists to calculate the forbidden energy band gap in simple periodic protein models^{2–7}. Conductivity measurements of several proteins under various conditions indicated a band gap or activation energy (E in the expression for the conductivity $\sigma = \sigma_0 \exp(-E/2kT)$ as function of the temperature T) of the order of 3 eV (refs 8, 9), a value in agreement with the only theoretical energy band gap available at that time (ref. 2). Therefore, the intrinsic mechanism of semiconduction along hydrogen bridges seemed to be well established in 1960. Later, however, both experimentally^{10–13} and theoretically the question arose whether the conduction mechanism is really intrinsic. The more refined all-valence-electron quantum chemical calculations^{5–7} indicated larger gaps (values in the range 6.1–16.7 eV were reported) than obtained in experiments. Most interestingly the calculation of Morokuma⁵ at complete-neglect-of-differential-overlap level showed that in a polyglycine chain the bandgap was not too sensitive to the changes of the chain's conformation: in conformers with appreciable H-bonding the gap found was slightly larger than in the β -sheet conformation. This latter finding pointed out the very small influence of H-bonds on the forbidden gap of periodic protein models. These semi-empirical calculation methods were designed

for molecular purposes and there is still little known of their applicability to the calculation of the gap in crystals and polymers^{14,19}. Thus we describe here use of the more reliable non-empirical ('*ab initio*') crystal orbital method¹⁵ to calculate the band gap in a most simple polypeptide model β -polyglycine.

We used an STO-3G atomic orbital basis set¹⁶. After evaluation of all integrals¹⁷, as usual in molecular *ab initio* calculations, we performed a self-consistent-field calculation using a first neighbour's interaction approximation. This highly sophisticated calculation resulted in a gap of 19.23 eV, the largest value obtained so far. Although larger numbers of included neighbours, basis set enlargement and correlation corrections may reduce this value by several eV, it seems impossible to reduce this value to the vicinity of the experimental gap. We may conclude therefore, that the intrinsic mechanism in a periodic protein model can be ruled out. Absolutely pure periodic proteins are thus good insulators. This conclusion will probably remain valid even in presence of orientational randomness of the constituent units. A set of aperiodic side chains, however, may serve as impurity centres, which support extrinsic type conduction.

The lowest energy optical transition of the electron system corresponds to the formation of excitons within the forbidden gap rather than to free electrons and holes. The order of magnitude of the energy of these excitons is expected to be 6–8 eV on the basis of the first electronic transition energies of small peptides¹⁸. Although this value is much larger than the experimental E it is necessarily smaller than the theoretical band-to-band gap found in our calculation.

Some technical details, which are of interest to specialists only, will be given elsewhere.

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Errata

The caption for the cover picture for *Nature*, vol. **264**, no. 5583 should have read: Microtubules polymerised *in vitro* and stained with the PAP-procedure with omission of the anti-tubulin antibody solution.

In the Statement published in *Nature*, vol. **265**, p. 764, the first line of the last paragraph of the letter from B. Hamprecht should have read 'The data on cyclic GMP were'.

In the article 'Low temperature specific heat of glasses and amorphous solids' by P. R. Couchman, C. L. Reynolds, Jr. & R. M. J. Cotterill, *Nature*, **264**, 534 (1976), the units for G in paragraph three are dyn cm^{-2} , not dyn cm^{-3} .

matters arising

Estimation of heritability from IQ data on twins

IN a recent paper¹ a nationally representative sample of twins born in one week of March 1958 was investigated to estimate kinship correlations in mental test performance for monozygotic and dizygotic twins. The authors concluded that their study offered "supportive evidence for zero or low upper limit heritabilities of mental test performance". We have received several critiques of the paper which we publish below, together with a response from the authors.

ADAMS *et al.*¹ have used a method² of estimating heritability, that is not reliable. The formula $h^2 = (r_{MZ} - r_{DZ}) / (1 - \rho_{00})$ was stated by Jensen³, without any theoretical justification, to measure heritability. In this formula r_{MZ} and r_{DZ} are the phenotypic correlations between monozygotic and dizygotic twins, respectively, and can be observed directly, but ρ_{00} , the genetic correlation between sibs, cannot be observed. It can be obtained from their phenotypic correlation if heritability is known, which, of course, it is not.

To overcome this difficulty, Jensen³ suggests that the formula $\rho_{00} = (1 + \rho_{pp}) / (2 + \rho_{pp})$, where ρ_{pp} is the genetic correlation between mates should be used to find ρ_{00} . He gives Li³, chapter 13, as reference for this formula. However, it does not appear there, and Professor Li informs me (personal communication) that he had never seen it before I brought it to his notice. This formula seems to have no theoretical justification. Moreover, it does not resolve the problem of finding genetic correlation. We now require the genetic correlation between mates which can be obtained from their phenotypic correlation if heritability is known. Thus, to use Jensen's formula for finding heritability of a trait, an estimate of that heritability is required.

Jensen³ resolves the dilemma by assuming that the genetic correlation between mates is 0.25, without telling his readers how this figure is obtained from their phenotypic correlation of 0.6 (ref. 4). The value of ρ_{DZ} , actually ρ_{00} , which Adams *et al.*¹ use is based on this assumed genetic correlation. The value of 0.8 for the broad heritability of IQ which is often quoted by Jensen and others is also based on this value. It is difficult to find a scientific justification for the method or

to have any faith in estimates of heritability based on it.

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THE report by Adams *et al.*¹ is non-contributory to the estimate of heritability of mental test performance for three reasons:

(1) No adequate information is given on the zygosity of the twin pairs. With a small sample (<100 like sex pairs) there would have been little difficulty in testing the twins for genetic markers such as blood groups, or at least having the twins examined by an experienced human geneticist, with special tests on those difficult to assign. It is true that a competent questionnaire to the parents will enable the correct type of twinning to be established in some 90% of cases. But merely to ask the parents their opinion is unreliable. Parents tend to be influenced by what they are told in the neonatal period by the midwife or obstetrician who usually erroneously assume that dichorionic twins must be DZ. Any errors in the assignment of the type of twinning will lead to an underestimate of heritability by the twin comparison method.

(2) An estimate of heritability based on the difference between the intra-class correlations of MZ and DZ twins is confounded by any substantial degree of assortative mating of the parents for the character in question. Insofar as any parental resemblance is genetic this will raise the DZ correlation, but not the MZ correlation, and so lower the estimate of heritability below the true value. Assortative mating for intelligence test score in most surveys is high, about 0.5. Adams *et al.* claim that the effect of such assortative mating on the estimates of heritability is likely to be small. This is not correct. For example, on the simple model of purely additive inheritance, with 100% heritability of intelligence test score, and 0.5 genetic parental correlation, the expected genetic DZ correlation is not 0.5 but 0.75. To

assume the value of 0.5 would give an h^2 of only 50% and not 100%. Jensen's quoted estimate of an expected DZ correlation of 0.55 seems implausibly low. It is the difficulty of estimating the expected genetic DZ correlation that make twin comparisons an unsatisfactory method of estimating heritability for any character for which there is substantial assortative mating. (3) Estimates of h^2 from twin correlations based on only 140 pairs have a large sampling error.

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¹ Adams, R., Ghodsian, M. & Richardson, K. *Nature* 263, 314-316 (1976).

ADAMS *et al.* estimate¹ of an upper limit of 0.373 is based on a sample consisting of 41 MZ and 95 DZ twin pairs. This estimate is subject to sampling error, and if one calculates a 95% confidence interval for the population value of the heritability one obtains an interval ranging from about zero to over 0.6. An exact interval is difficult to establish, but using an estimated s.e. of about 0.2, the value of 0.6 can be regarded as a conservative upper limit.

Thus, while the data the authors present are an interesting contribution to this subject and do not seem to support a heritability value as high as 0.8, the assertion of a 'low' upper limit is not really justified.

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¹ Adams, R., Ghodsian, M. & Richardson, K. *Nature* 263, 314-316 (1976).

It is perhaps surprising that the authors of a study¹ comparing 'mental abilities' in MZ and DZ twins should place such reliance on tests which are not validated, and were administered in a group situation, where contamination effects are difficult to control. If, as is probable, pairs of same-sexed twins sit next to one another in a classroom during the test, it is perhaps

quite likely that they will achieve similar results.

An important but neglected study of twins by Claridge *et al.*² seems to have avoided at least some of the methodological difficulties which faced the National Children's Bureau study. Claridge's study compared 44 MZ and 51 DZ twin pairs, aged 16–55, resident in Glasgow. Zygosity was established through comparative blood grouping. Among the battery of tests completed by the subjects were the Progressive Matrices and the Mill Hill vocabulary test, well-established and validated measures of mental ability, individually administered.

The intraclass correlations for MZ pairs on the Progressive Matrices was 0.68, and for the DZ pairs it was 0.46. On the Mill Hill vocabulary test, the intraclass correlation in MZ pairs was 0.85, compared with 0.68 in the DZ pairs. For both tests, the intraclass correlation was significantly higher in MZ pairs. Although the authors do not discuss the implications which these results have for models of the inheritance of 'intelligence', it is clear that these results imply that at least some of the variance in mental abilities in this adult Scottish sample is due to inheritance.

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¹ Adams, B., Ghodsian, M. & Richardson, K. *Nature* 263, 314–316 (1976).

² Claridge, G., Canter, S. & Hume, W. *Personality Differences and Biological Variations: A Study of Twins* (Pergamon, Oxford, 1973).

THE report by Adams *et al.*¹ of correlations between mental test scores of MZ and DZ twins raises questions about the variance of obtained correlations in twin studies which were not discussed by the authors. Instead, they used their data to suggest the dismissal of previous estimates of heritability; but if the figures are considered in relation to other findings more complex issues emerge. These are the questions of how the various samplings stand as bases for estimating the same population parameter, and of what other features of the testing and sampling (apart from the well known difficulties of mental testing and the identification of twin types) may contribute to the variance of sample correlations.

The picture of twin study findings is represented in Table 1, where Adams' figures are compared with corresponding results from an earlier well-founded report², and with others from the review by Erlenmeyer-Kimling and Jarvik³ which gave figures from 34 studies concerned with twins. The

Table 1 Correlation coefficients in the mental testing of twins

Source	MZ		Twin type (all reared together)					
	r	N	Same sex		Opp. sex		All	
			r	N	r	N	r	N
Adams <i>et al.</i> (non-verbal group test, 1969)	0.762	41	0.604	55	0.487	40	0.594	95
Newman <i>et al.</i> (group test, 1937)	0.992	50	—	—	—	—	0.621	51
Erlenmeyer- Kimling <i>et al.</i> (median values of 34 studies, 1963)	0.87	1082 (in 14 studies)	0.53	11 studies	0.53	9 studies	0.53	2052 (in 20 studies)
Erlenmeyer- Kimling <i>et al.</i> (1963) upper limit	0.92		0.87		0.63		0.87	
Erlenmeyer- Kimling <i>et al.</i> (1963) low limit	0.76		0.45		0.37		0.37	

Adams figure for MZ twins lies at the lower limit of previous studies, while the figure for DZ twins lies in the upper part of the range. Thus that particular sampling must yield one of the smallest, if not the smallest, difference between such correlations in the whole series of studies. Table 2 shows the statistical

for their study is likely to be either a rather extreme sampling or evidence of a change in a population characteristic. Rather than discussing the implications for estimates of heritability it is important therefore to consider the possibility of change in the population correlation figure for MZ twins.

Table 2 Differences between correlations for MZ and DZ twins

Statistic	Adams		Source Newman		Erlenmeyer- Kimling	
	MZ	DZ	MZ	DZ	MZ	DZ
r	0.762	0.594	0.922	0.621	0.87	0.53
Fisher z difference	1.000–0.683		1.605–0.727		1.333–0.590	
σ_z difference	0.193		0.205		0.037	
Ratio	1.642		4.283		20.081	
Significance level	Not sig.		0.01		0.01	

significance of the differences. While the Newman figures are not exceptional in this respect, the Adams figures clearly are.

In Table 3, figures are compared across the studies for both kinds of twins. For DZ twins none of the differences are statistically significant, even

Since available twin studies span a considerable time, during which advice has been given to parents to treat identical twins as individual persons, this may have had the effect of increasing the within-pair variance. More sampling is necessary to test the present position further, as is a thorough

Table 3 Differences between corresponding correlations in different studies

Statistic	Newman–Adams		Difference Newman– Erlenmeyer- Kimling		Erlenmeyer- Kimling–Adams	
	MZ	DZ	MZ	DZ	MZ	DZ
Fisher z difference	0.605	0.044	0.272	0.137	0.333	0.093
σ_z difference	0.218	0.178	0.149	0.146	0.165	0.107
Ratio	2.775	0.247	1.825	0.938	2.018	0.869
Significance level	0.01	Not sig.	Not sig.	Not sig.	0.05	Not sig.

at the 0.05 level, and the samples could be treated as coming from the same population of such twins; but for the MZs the pattern is such that, whereas the Newman and Erlenmeyer-Kimling samples could be treated as bases for the estimation of the same population correlation, it is doubtful whether the Adams sample can be so regarded.

This being so, it seems that Adams and his colleagues may have been hasty in offering their findings simply as evidence for a low heritability estimate,

analysis of data to test the hypothesis of a trend over time.

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¹ Adams, B., Ghodsian, M. & Richardson, K. *Nature* 263, 314–316 (1976).

² Newman, H. H. *et al.* *Twins: A Study of Heredity and Environment* (University of Chicago, 1937).

³ Erlenmeyer-Kimling, L. & Jarvik, L. F. *Science* 142, 1477–1478 (1963).

ADAMS *et al.* reply—None of the above criticisms substantially alters our conclusions, though some underline the caution appropriate in certain places.

Dr Vetta has to be thanked for showing that assumptions made by Jensen in deriving a formula for heritability estimation are without theoretical justification. It follows that the formula ought not to be perpetuated. As regards the implications of this for our own report we should like to make three points: (1) We calculated heritability both with and without Jensen's false assumptions; (2) the calculation itself (we made a point of saying) was the demonstration of an *erroneous* estimation by incorporation of 'treatment effects'; (3) our main conclusion was based on the comparison of raw correlations.

Carter underestimates the generally accepted level of accuracy of zygosity determination in twins by impressionistic methods. Such methods are routinely adopted, especially under difficult conditions such as the geographic dispersal of subjects. Vandenburg¹ has claimed in a review of the approach, that "it has been shown repeatedly that for large-scale twin studies a few questions about the frequency with which close friends or relatives mistake one twin for the other will provide a sufficiently accurate diagnosis within the limits of the accuracy of the variable under study whether it be a mental test score or a physical illness". Cederlof *et al.*², found 98% agreement with blood-typing for responses on one of their items on a mailed questionnaire.

Carter claims, however, that merely asking mothers is too unreliable because they may have been confused by neonatal misinformation. This is quite plausible, but we need evidence about the extent of confusion, especially many years after the births. Cohen *et al.*³ mailed a questionnaire to 35 mothers of twins in their Louisville twin study. They asked them (among other things) whether the twins were identical or fraternal, and twins were subsequently blood-typed. In only two cases did definite misclassification occur through neonatal misinformation, and there were no other misclassifications. Hence their assertion that (apart from the above) "we did not find any mother who seriously believed that her MZ pair was DZ, nor any mother of DZ twins who believed that her twin pair was MZ". Their general conclusion was that "parental perceptions of identical and fraternal twins were extremely different". The correlations for height at 7 yr in our sample (height being a trait of indisputably high heritability) were: $MZ=0.926$; $DZ_{ss}=0.546$. We thus do not believe our twin classification was too unreliable though,

naturally, caution would not be unwarranted.

Carter illustrates how non-correction for assortative mating could underestimate h^2 . The illustration depends, however, on the prior assumptions of high h^2 and high genetic correlation of mates. Both assumptions become less tenable as MZ and DZ correlations approach identity, as is the case in our sample. He does not explain, moreover, why Jensen's estimate of $pDZ=0.55$ "seems implausibly low". The simple fact is that values higher than that applied to previous twin correlations yielded impossible h^2 values >1.00 . Both forms of special pleading (high Δr_s implying low pDZ ; low Δr_s implying high pDZ) necessary to preserve $h^2=0.8$ indeed suggests a limitation of the method, but this does not diminish the finding of insignificant Δr_s as an empirical contribution. Our sample size was comparable with (if not larger than) previous studies; bigger ones are unlikely to be forthcoming without transgressing very stringent sampling conditions, satisfying which was our study's main strength.

Harvey Goldstein quotes our conclusion, based on comparison of MZ and DZ_{ss} correlations, and then claims that our "heritability estimate of 0.373" is consistent with an upper confidence limit of 0.6. It should be clear from our paper, however, that that estimate is based on MZ and DZ_{ss} correlations, for non-verbal correlations only, calculated purely to illustrate the inflating consequences of introducing 'treatment effects'. It would be quite indiscriminate to transpose that h^2 value and its 'guesstimated' confidence limit to MZ- DZ_{ss} differences which (1) are not significantly different, (2) are quite different as regards verbal and non-verbal measures, and (3) probably themselves contain treatment effects. Our conclusion of "supportive evidence for zero or low heritabilities" seems to us a more circumspect one.

Bagley seems vague about test validity. The test we used has received some validation and a reference was given. It has been administered to two very large nationally representative samples of 11-yr olds so that excellent information is available about its distributional properties and correlations with other measures, teachers' impressions and so on. It should be pointed out that there is no universally validated and standardised intelligence test in this country, which explains the interest in the long-gestating British Intelligence Scale. As well as face-validity, ultimate validation is always by school attainments/teachers' perceptions; the items in our test conformed closely to the half dozen or so types used in conventional intelligence tests⁴. Bagley is quite wrong to uphold

the Claridge *et al.* study as a model. For example, vocabulary test is not a usually accepted intelligence test and the Progressive Matrices has been far from satisfactorily validated and is also a group test. Moreover, it is a test for subjects of '11 years plus', whose use on very much older people for the purpose in mind is dubious. It is the problem of testing such a wide age-range of twins (16-55 yr) especially if the ranges for MZ and DZ twins are quite different, that has flawed previous studies and makes ours (of uniform age) unique. Furthermore, the small number of DZ pairs in the Claridge *et al.* study did not allow separation of opposite-sex and same-sex twins so that their (significant) correlation differences could quite easily be explained by the sort of 'treatment effects' which our own study suggests. Finally, the contamination effects of which Bagley speaks, relating to same-sex twins, are as applicable to MZ as to DZ twins, making separation of DZ_{ss} and DZ_{os} correlations all the more desirable.

Francis is wrong to think that we used our data primarily to suggest the dismissal of previous estimates of heritability. It is the doubts about the empirical sufficiency of previous estimates which have suggested their dismissal. Accordingly, it seems quite wrong to treat our findings as co-extensive with previous ones as if they were of equal empirical sufficiency. Again the Newman, Freeman and Holzinger study sample twins of a very wide range of ages and their small numbers of pairs did not allow separation of the DZ_{os} and DZ_{ss} pairs. Similarly, the well known Erlenmeyer-Kimling and Jarvik study includes studies of widely different measures, many with very small samples, wide age ranges, etc. Thus we dispute Francis's inference that our results, from a nationally representative group of the same age, can be explained away by 'extreme sampling'; indeed, speculation of that sort seems a pointless exercise. Of course a changing 'population characteristic', and a 'labile heritability', is a possibility, but debatable only with comparable studies; the studies of Cohen *et al.*³ and several others, moreover, suggest that identical twins are still treated much more similarly than are fraternal twins.

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¹ Vandenburg, S. G. in *Genetics* (ed. Glass, D. C.) (Rockefeller University, 1968).

² Cederlof, R. *et al. Acta genet. basel.* 11, 338 (1961).

³ Cohen, D. J. *et al. Archs. gen. Psych.* 29, 465 (1973).

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⁵ Vernon, P. E. *Intelligence and Attainment Tests* (University of London, 1966).

Mechanism of stomatal movement

ACCORDING to Bowling's malate-switch hypothesis¹ of stomatal action, the pH changes in the guard cell create a gradient of monovalent malate towards either guard cells or epidermal cells causing a diffusion of K⁺ (bound to monovalent malate) along with the gradient. His theory is based on (1) changes in pH of guard cells during opening or closure, (2) balancing of K⁺ by the amounts of malate and chloride present in epidermal tissue, and (3) no change in the net amounts of K⁺, Cl⁻ or malate in epidermal tissue.

Changes in the pH of guard cells do occur^{2,3}, however. Malate and Cl⁻, together, can balance K⁺ present in guard cells⁴⁻⁶. There is also no net change in K⁺ of epidermal tissue⁷. The replaceability of K⁺ by Na⁺ for stomatal opening demonstrated that a monovalent ion is necessary to increase the turgor pressure of guard cells but not necessarily K⁺ (refs 8-10).

We cannot reconcile these data on malate with the proposals of Bowling¹. The epidermal tissues produce appreciable quantities of malate and the changes in malate levels are closely and positively correlated with the stomatal aperture^{5,6,11}. Further, the epidermal tissues contain highly active enzymes not only for malate synthesis but also for malate degradation^{12,13}. The above data, together with the autoradiographic studies indicate that malate production and metabolism could occur in guard cells^{2,11-13}. The general understanding is that guard cells can derive malate through phosphoenolpyruvate carboxylation and may exchange those acid ions for K⁺. The stimulation of stomatal opening by phosphoenolpyruvate¹³ supports this assumption.

In fact, no anion is required for K⁺ uptake by guard cells since the guard cell can itself release H⁺ ions¹⁴. We therefore feel that, in addition to malate, photo-synthetic and/or respiratory electron transport-mediated H⁺/OH⁻ efflux could cause a change in the pH and provide the necessary H⁺ ions for exchange with K⁺. We have presented evidence for such light-induced efflux in guard cells¹⁵. An active uptake of K⁺ is likely because the stomatal response to even CO₂ free air is sensitive to metabolic inhibitors¹⁶. Absciscic acid also interferes with energy metabolism in guard cells^{17,18}. Active K⁺ transport is, however, not ruled out by Bowling¹.

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- ¹ Bowling, D. J. F. *Nature* **262**, 393-394 (1976).
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BOWLING REPLIES—In developing the malate-switch hypothesis I considered the evidence for a connection between overall malate levels and stomatal aperture quoted by Das, Rao and Raghavendra¹. I do not think, however, that the evidence is as strong as they imply. For example, Pearson and Milthorpe² showed that with

much more rapid than do changes in overall levels of organic acids and other metabolic products.

I put forward the malate-switch hypothesis after realising that there is a transport dimension as well as a metabolic dimension to stomatal functioning. A hypothesis which assumes that stomata open as a result of synthesis of malate in the guard cells and subsequent exchange of H⁺ ions with K⁺ has also to explain how K⁺ moves to the guard cell from three cells away as it does in *Commelina communis*. An accompanying anion is surely required and on present knowledge this would seem to be malate.

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- ¹ Das, V. S. R., Rao, I. M. & Raghavendra, A. S. *Nature*.
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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

a malate concentration of 5 µg mg⁻¹ the stomatal aperture ranges from 4 to 24 µm. Allaway³ qualifies his conclusions by stating that his results were obtained after 3-h treatments in light or dark, and questions whether the malate-aperture correlation exists in other conditions. Willmer and Dittich⁴ found no difference in the rate of incorporation of ¹⁴C into malate in the light or the dark by *Commelina diffusa*. They point out that changes in stomatal aperture seem to be

Scrotal asymmetry

FOR the record, I report that since reading Morgan and Corballis¹ on the subject of scrotal asymmetry and Rodin's dyslexia, I have made regular observations of the asymmetry in one person who is right handed, has not got situs inversus totalis (or even partialis), has never had surgical procedures on the abdomen, and has no neurological, endocrinological or psychological disorder, except a certain impatience with pretentious nonsense. My findings are that if the subject of my investigation had been the model for Rodin's *L'Age d'Airan*, Rodin would have been correct in assigning a lower position to the right testis compared with the left. I am not dyslexic.

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This correspondence is now closed. Ed.

reviews

Caricature of Darwinism

R. C. Lewontin

The Selfish Gene. By Richard Dawkins. Pp. xi+224. (Oxford University; London, 1976.) £2.95.

POWERFUL theories that offer to explain large parts of the world of appearances carry, immanent within them, their own caricatures. Indeed, their liability to absurd reduction is precisely in proportion to their original generality and power. There are inevitably those who, dazzled by the great insights of others but understanding them only superficially, push these theories far beyond their valid domain of explanation. This has been the fate of the three powerful conceptual systems that have had such an immense impact on the life and thought of the nineteenth and twentieth centuries: Marxism, Freudianism and Darwinism. Marx's historical materialism has been vulgarised by an 'economism' that attempts to explain the last petty detail of social organisation and history by direct reference to 'material conditions'. Carried away by Freud's theories of the unconscious, and especially the transformations of behaviour described as repression, inversion and sublimation, facile 'psychologism' has searched for an explanation of the French Empire in Bonaparte's relationship with his parents.

The caricature of Darwinism which sees the wonderful workings of natural selection in every aspect of the living world and its artefacts, began immediately with the publication of the *Origin of Species*. The enthusiasm that led the early partisans of Darwin to see every difference in the morphology and physiology of organisms as adaptive, also led them into speculation no different from the baroque rationalisation of Paley's *Vestiges of Creation*. Evolutionary theory was rescued from its excesses by Huxley's ideas of allometric growth which enabled us to understand that many morphological features of organisms are simply epiphenomenal consequences of relative changes in different growth fields or in different dimensions, and by Sewall Wright's demonstration that precisely the same selective forces can lead different populations to quite different phenotypic compositions because of random processes operating in a field of multiple stable equilibria. Thus, we

no longer feel called on to explain in an adaptive mode why the Indian rhinoceros has one horn whereas the African rhinoceros has two.

For more than forty years evolutionary theory has remained free of a naive selectionism, but in recent times there has been a return to the extreme form of the adaptationist program, as evolutionists have rediscovered behaviour. Beginning with the undoubted truth that behaviour must, like morphology and physiology, be subject to the force of natural selection, the new Panglossians end with the old error that *all* describable behaviour must be the direct product of natural selection. The scientific manifestation of this trend can be seen in every issue of say, *The American Naturalist*, which is permeated by the language, if not the formal apparatus, of game theory, and in the development of the school of 'sociobiology', among whose more extraordinary productions is a recent highly praised dissertation explaining *fellatio* and *cunnilingus* among the upper middle classes as an adaptive response to constant resources. The popular manifestation of this new caricature of Darwinism reaches its most extreme form in *The Selfish Gene* by Richard Dawkins.

The Selfish Gene is the result of Dawkins' discovery of vulgar Darwinism. "We are survival machines—robot vehicles blindly programmed to preserve the selfish molecules known as genes. This is a truth which still fills me with astonishment" (preface). I too find Dawkins' description of the relationship between gene and organism rather astonishing. The theme of our passive manipulation by our gene captors is repeated over and over. "They swarm in huge colonies, safe inside gigantic lumbering robots, sealed off from the outside world communicating with it by tortuous indirect routes, *manipulating it by remote control*. They are in you and me; they created us *body and mind*; and their preservation is the ultimate rationale for our existence. They have come a long way, those replicators. Now they go by the name of genes, and we are their survival machines" (p21). "It leaps from body to body down the generations, *manipulating body after body* in its own way and for its own

ends, abandoning a succession of mortal bodies before they sink into senility and death" (p36) (my italics throughout).

The form of reasoning that leads Dawkins, or, rather, the evolutionary theorists of whom Dawkins is the enthusiastic reporter, to this view of gene and organism is exemplified in the discussion of crowding and fertility. It is observed that in some animals overcrowding reduces the birth rate. Given this observation, Dawkins then asks "Why does natural selection favour females who reduce their birth rate when the population is overcrowded?" He then contrasts a population selection argument, unfavourably, with a "selfish gene" theory. But both arguments assume without proof that females reduce their birth rate for an adaptive reason, rather than regarding lower fertility as a mechanical concomitant of the altered endocrine function that results from excitement. They also assume that at some time in the evolutionary past the ancestors of these animals did *not* respond to crowding by lower fertility and that genetic variation of just the right sort conveniently arose to allow some animals to do so. Finally, they invent a plausible story to explain why lowered fertility is a 'good thing'. With a little ingenuity, of course, such stories can always be invented, but it is not clear whether we are dealing with science or high-table wit. The realisation that he is engaged in game playing after all comes briefly to Dawkins' consciousness in his discussion of R. L. Trivers's story of reciprocal altruism. "There is no end to the fascinating speculation which the idea of reciprocal altruism engenders when we apply it to our own species. Tempting as it is, I am no better at such speculation than the next man, and I leave the reader to entertain himself" (p202).

Dawkins is not content that his readers should amuse themselves with the clever games already produced by Hamilton, Trivers and Zahavi, but introduces, in his last chapter, his own patented variant. Allowing that the details of human culture are probably not coded in our genes (we are not such robots, after all, it seems), Dawkins suggests that ideas themselves are units of reproduction and natural

selection. He asserts, for example, that "The idea of hell-fire is, quite simply, *self-perpetuating*, because of its own deep psychological impact" (p212), although he does not tell us how its "deep psychological impact" provides a mechanism for its self-perpetuation. He shrugs off without analysis the much more plausible and clearly causal hypothesis that hell-fire is not *self-perpetuating*, but is perpetuated by some people because it gives them power over other people. In fact, both Dawkins' suggestion that ideas are replicating selected units and the sociologists' notion that the details of human social activity are coded in our genes and selected for maximum reproductive potential, arise from the same fallacious view of human society, which, in turn, is a reflection of their confusion between materialism and reductionism. Although it is true that human beings are material objects whose brains are the

result of a developmental process under the influence of genes, it is not true that their *minds* can be understood when we understand their genes. In like manner, although human society is the product of the sensuous activity of material individuals, human society is not simply the collection of all individuals.

According to Richard Dawkins, "If superior creatures from space ever visit earth, the first question they will ask, in order to assess the level of our civilisation is 'Have they discovered evolution yet?'" I think it more likely they will want to know whether we know the difference between properties of sets and properties of their members. □

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Compendium of physiology

Introduction to Physiology. Vol. 3. By H. Davson and M. B. Segal. Pp. x+656. (Academic: London; Grune and Stratton: New York, 1976.) £9.80.

THE appearance of the third volume of this series so shortly after the first two (for review see *Nature* **259**, 428; 1976) is a great tribute to the industry as well as the erudition of the authors. It is a pity, even so, that they have been outpaced by inflation and the cost per page has risen from about 13p to 15p.

This third volume is devoted mainly to the homeostatic control of the principal mass-transport systems of the mammal—the circulatory, respiratory, alimentary and urinary systems. There are also sections, in the Bernard tradition, on thermoregulation, the control of plasma electrolytes and blood sugar, and a concluding one on the internal environment of the central nervous system in which the writers are, of course, very much on their home ground. Each of these topics is covered in considerable depth and the bibliography which is appended to each section is copious including virtually all workers who have achieved prominence in their respective fields over the past 20 years as well as many whose contributions are less familiar.

What seems to be emerging is a compendium of contemporary physiology rather than an introduction to the subject. The various sections, although providing detailed and thoughtful surveys of current ideas, suffer somewhat from a lack of contour lines which might help the noviti-

ate to distinguish the salient features in the detailed scenario which is presented. The text is complemented by frequent clear and apposite illustrations, although in one instance, at least, the figure contradicts what is written elsewhere and occasional pictures of apparatus hardly merit the space they occupy. In the text itself there are some confusing and inconsistent statements but these are rare and could well result from hasty proof-reading. Also from time to time an idea is tantalisingly floated without being adequately developed.

Now that the half-way stage in its production has been reached, the question must arise as to what type of user is likely to gain most from this multi-volume treatise. My own impression is that it is too detailed to serve as a working source book for the average medical student who may, however, find it valuable for occasional consultation. On the other hand, Honours undergraduates in most UK universities would probably be encouraged to read original papers rather than rely on summaries, however well prepared. This leaves postgraduate students who, particularly on taught courses, could find their needs well catered for, and finally there is the harassed teacher who may be called on to give lectures or tutorials outside his own speciality. Persons in the last category should be heartily grateful to Drs Davson and Segal for having so diligently 'researched' such a wide range of important topics and having so skilfully blended and packaged the fruits of their labours.

R. V. Coxon

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Isotachophoretic analysis

Isotachophoresis: Theory, Instrumentation and Applications. (Journal of Chromatography Library, Vol. 6.) By F. M. Everaerts, J. L. Beckers and T. P. E. M. Verheggen. Pp. xiv+418. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl. 160; \$61.50.

THIS is an important book. Its senior author, Dr F. M. Everaerts, was introduced to isotachophoresis some twelve years ago by his PhD supervisor, the legendary A. J. P. Martin. Since then Everaerts and his group have developed the technique from a laboratory curiosity to arguably the most versatile method available for the characterisation and determination of low to medium molecular weight electrolytes. It is a method which in my view is destined to become one of the standard tools of chemical analysis.

Essentially the book is a full report of the work done by Everaerts' group; as such the treatment of the subject is uneven. The theoretical section does not provide a good introduction to the subject. The long chapter describing a mathematical model of isotachophoresis seems out of place and adds little of practical value. The qualitative aspects of isotachophoresis, however, are adequately described and the chapter on ionic mobilities summarises the variables available for exploitation by the analyst.

The section on instrumentation is by far the best. It provides a clear account of the problem of detection and of presently available solutions to it. The descriptions of the apparatus are complete enough for anyone with sufficient skill to build an analyser for himself.

The applications section is the most disappointing of all. In spite of the wealth of experimental data presented, there are no comparisons made with rival, well established techniques. A careful perusal of the separations depicted in (say) Fig. 12.4 (p 303) and Fig 15.1 and 15.2 (pp 350–351) will convince any analyst of the value of the technique.

In spite of its shortcomings this book ought to be read by all analysts of electrolyte solutions. Scientific instrument manufacturers should also find it of considerable interest, and possibly very profitable.

Frank Hampson

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Carcinological fauna of Japan

Crabs of Japan and the Adjacent Seas. By Tune Sakai. Volume 1: English text+figs. Pp. i-xxix+772. Volume 2: Plates. Pp. 250. Volume 3: Japanese text. Pp. 460. (Kodansha: Tokyo; Antiquariaat Junk: Lochem, The Netherlands, 1976.) Dfl.550 the set.

DURING the past two decades research on the carcinological fauna of Japan has made rapid progress, resulting in many additions to the species of crabs. The author of this handsomely produced, up-to-date, and definitive monograph has kept abreast with the recent literature and incorporated the results of over forty years of field work and research. Considerable changes in classification and nomenclature have therefore been made—for example, to the family Potamonidae after Bott, 1970; and to the genus *Uca* after Crane, 1975.

About 885 species of Brachyura, referable to 329 genera and 30 families, are dealt with taxonomically, and nearly all are illustrated. Numerous keys are given to the determination of most categories from superfamilies to subspecies. Exceptions are, for example, species of *Ebalia*, which require revision; and genera in the Alliance Xanthoida, because Mme Guinot's investigations are still in progress. Well known species, or ones described in the earlier monograph,

or in *The Crabs of Sagami Bay* (1965), are not described again.

Throughout, references and synonyms, material examined, habitat with occasional ecological notes, type locality and general distribution, are given. Mention is made of some recently described species not seen by the author. Quite a large percentage are endemic; a few are known only from Japan and some remote locality like Ceylon, or Madagascar. Many are widely distributed throughout the Indo-West Pacific.

Because of the great economic importance of some of the cold water crab-like Anomura of the family Lithodidae, fifteen species (two new to science) are described and illustrated in an Appendix. Bibliographies for the Brachyura and the Lithodidae, and a systematic index, complete the English text. The author's English is always easy to follow, and the Japanese text is apparently intended for the use of students and non-specialists.

The colour plates are excellent. About 600 beautiful paintings after living or frozen crabs, specially prepared by the author's wife, are reproduced; and a number of rather striking colour photographs of crabs in their natural habitats are included. The book will be useful for specialists working on the fauna of the whole of the Indo-Pacific region.

Isabella Gordon

Isabella Gordon recently retired after many years as Head of the Crustacea Section at the British Museum (Natural History), London, UK.

ISIS bibliography

ISIS Cumulative Bibliography: A Bibliography of the History of Science formed from ISIS Critical Bibliographies 1-90, 1913-65. Vol. 3; Subjects. Edited by M. Whitrow. Pp. 678. (Mansell Information: London, 1976.) £28.

THIS massive work brings one stage nearer completion a major scholarly project reviewed in these pages five years ago (*Nature*, 237, 115-116) when the initial two volumes were published. The *ISIS Cumulative Bibliography* aims to make accessible the rich information on "the history of science and its cultural influences" stored in the critical bibliographies routinely published by the official journal of the History of Science Society. Volumes 1 and 2 recovered and reorganised material under the headings of "personalities" and "institutions". This third volume deals with "subjects". "Periods" will occupy a fourth volume.

The difficulties inherent in this kind of bibliographical synthesis are commensurate with its magnitude. The abbreviations for the journals cited run to seventy-

six closely-printed quarto pages. The classification scheme needs ten pages of explanation. That scheme allows the more than twenty thousand entries to be arranged and cross-indexed under approximately seven thousand categories. In an interesting reflection of historical priorities, works on mathematics and the physical sciences occupy one hundred and twenty-three pages to a mere thirty for the biological sciences and twenty-nine for the sciences of man.

The present volume allows one quickly to identify the reliable historical literature on subjects ranging from "Adsorption: Chemistry" to "Alchemists: North American: Collective Biography", and "University and Advanced Level Teaching: Mathematics: Russia" to "Writing and Reviewing: Science". Like its predecessors, this volume is an indispensable reference work for all those concerned to understand science as a human enterprise.

Arnold Thackray

Arnold Thackray is Chairman of the Department History and Sociology of Science, University of Pennsylvania, and will be a Visiting Fellow at All Souls College, during 1977-78.

Underwater optics

Marine Optics. By N. G. Jerlov. (Elsevier Oceanography Series, No. 14.) Pp. xiii+231. (Elsevier: Amsterdam, Oxford and New York, 1976.) Dfl.77; \$29.75.

At first sight this book seems to be a glossier and larger version of Jerlov's *Optical Oceanography* (Elsevier Oceanography Series, No. 5, 1968); indeed the earlier book is still advertised on the dust cover of the present book, which seems a little strange. A closer look shows that the number of figures has increased from 83 to 137, tables from 28 to 37, but pages only from 173 to 203. Impressively, the references have increased from 296 to no less than 548, of which nearly 200 are post-1968. This certainly confirms the author's statement that progress in ocean optics is being made at a rapid rate.

In revising the book the author has eliminated some earlier references and included many others, as well as adding so much new work. In particular one should mention the large number of Russian and eastern European references (about 70) in the recent volume, compared with about 30 in the earlier one. The author's technique in revising has been mainly to repeat word for word the 1968 book, here and there inserting new paragraphs or tagging on bibliographical information. The extra 30 pages are mostly made up by the extra figures and tables.

It should be stressed there is almost no re-writing of the 1968 text and thus it becomes necessary to justify the production (and purchase) of the new volume purely on the extra information included. The price of about £18 does seem high but the eminence of the author and the assembling of so much extra material probably does justify such expenditure. The new data probably are better shown as figures and tables, but the main advances over the past eight years are more in the nature of additional data built on to old, rather than the formulation of new concepts.

One of the most valuable new sections deals with the use of underwater optics to check the movement of water masses, a valuable tool for the physical oceanographer. As in the 1968 version, however, the author disappoints the biologist reader who is looking for the application of underwater optics to such phenomena as primary production, bioluminescence and animal behaviour.

J. H. S. Blaxter

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GABA

GABA in Nervous System Function. (Kroc Foundation Series, Vol. 5.) Edited by Eugene Roberts, Thomas N. Chase and Donald B. Tower. Pp. xv+554. (Raven: New York, 1976.) \$30.00.

THE identification of γ -aminobutyric acid (GABA) in mammalian brain in 1950 led, after a short lag phase, to a profusion of physiological and biochemical studies. Many of these were described in the only previous volume on GABA and the nervous system, published in 1960. At that time the possible neuronal inhibitory role of GABA, and its importance in epileptic activity, were the focus of attention. A subsequent period of doubt was followed by one of consolidation. An inhibitory transmitter role for GABA in the mammalian cerebellum, cortex and hippocampus is now generally accepted. Experimentally, epileptic convulsions can be induced by drugs which block the synthesis of GABA or impair its inhibitory action. It has yet to be shown, however, that epilepsy in man (except for pyridoxine-related syndromes) arises from a failure in GABA-mediated inhibitory processes.

The present volume arises from a workshop held in California in 1975. The 37 chapters are by 47 contributors (32 from North America, 10 from

Japan, 2 from Norway, 2 from West Germany and 1 from Australia). The predominant emphasis is on biochemistry, cytology and cellular physiology. The last 5 chapters discuss the possible involvement of GABA in disease and therapy.

There are indications that we are entering a new growth phase in GABA research. Suggestions that abnormal functioning in GABA systems contributes to the pathogenesis not only of epilepsy, but also of Huntington's chorea, Parkinsonism, schizophrenia, senile dementia, and anorexia nervosa, are providing the stimulus.

The techniques and basic data presented in this volume provides the means. Of outstanding importance is the development of immunocytochemical techniques which, using antibodies prepared from purified enzymes (glutamic acid decarboxylase and GABA transaminase), enable visualisation of cellular structures containing these enzymes. Pioneering reports from the laboratory of Eugene Roberts, describe the light and electron microscopical localisation of these enzymes. These reports provide beautiful confirmation of neurophysiologists' earlier conclusions about which cell types release GABA in the cerebellum and hippocampus.

Brian Meldrum

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Polymerisation

Thermodynamics of Polymerization. By H. Sawada. Pp. xiii+403. (Dekker: New York and Basel, 1976.) 130 Swiss francs.

THIS book is an expanded version of the collection of articles written by Dr Sawada for *Reviews of Macromolecular Chemistry* over the period 1969 to 1974. Each chapter has been written as a separate entity so that there is inevitably a certain amount of repetition of both content and references.

The first two chapters are introductory, dealing with the general ideas of ceiling temperature, heats and entropies of polymerisation. These are followed by five chapters covering in succession anionic, cationic, radical, condensation and ring polymerisation. Next comes a chapter on polymerisations, the equilibrium positions of which have been measured, followed by two on copolymerisation. Finally there are two chapters devoted to degradation of polymers and miscellaneous topics such as tacticity and pressure effects.

The reader who comes to the subject afresh may be surprised to discover how much of the book is concerned with

kinetics rather than thermodynamics. A more complete description would have been "Kinetic and thermodynamic aspects of processes relevant to polymerisation". Thus under anionic polymerisation we find a discussion of equilibrium between different types of propagating species, under cationic polymerisation an account of the thermochemistry of ionisation processes, and under radical polymerisation substantial sections on the thermodynamics of the initiation, termination and transfer reactions. The kinetic data are sometimes linked with thermodynamically-based parameters such as Hammett σ , and sometimes simply expressed in terms of energies, entropies and volumes of activation. Other times there is only the most tenuous link with thermodynamics, as in the discussion of reactivity ratios in terms of the Alfrey-Price Q, e scheme.

The chief merit of the book is that it brings into a single volume for the first time most of the available information on thermodynamics of polymerisation, and a valuable feature is the Addendum of recent publications.

K. J. Ivin

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Wax esters and non-polar lipids

Chemistry and Biochemistry of Natural Waxes. Edited by P. E. Kolattukudy. Pp. xx+464. (Elsevier: Amsterdam, Oxford and New York, 1976.) Dfl129; \$49.75.

THIS book is dedicated to Professor A. C. Chibnall who first became interested in waxes fifty years ago and (with S. H. Piper) carried out some classical studies in this field. Significant advances in our understanding of wax chemistry and biochemistry had to await the chromatographic and spectroscopic procedures which have revolutionised and activated all areas of lipid research. The waxes (broadly interpreted) have shared in these developments, and for any who have overlooked them the record is fully documented in this book. Chemists and biochemists interested in lipid methodology, lipid structure, and lipid biosynthesis, and biologists of many kinds will want to consult this book and to have frequent access to it.

The term "wax" is not here confined to wax esters but includes a wide variety of non-polar lipids: hydrocarbons, ketones and aldehydes, primary and secondary alcohols, ethers, alkanediols, acids, wax esters of several kinds, and terpenoids. In general these may be saturated or unsaturated and straight chain or branched chain. The editor has chosen, however, to organise the chapters on the basis of the type of organism from which the wax is derived, the ten contributions being: introduction to natural waxes (P. E. Kolattukudy); mammalian waxes (D. T. Downing); marine waxes (J. R. Sargent, R. F. Lee and J. C. Nevenzel); bird waxes (J. Jacob); and their biochemistry (P. E. Kolattukudy); insect waxes (L. L. Jackson and G. J. Blomquist); plant waxes (A. P. Tulloch); and their biochemistry (P. E. Kolattukudy, R. Croteau and J. S. Buckner); algal and fungal waxes (J. D. Weete); and bacterial waxes (P. W. Albro). Dr Kolattukudy is to be congratulated on securing the service of such authoritative contributors. In addition to his own writing the editor has—more or less successfully—prevented undue overlap of material between the various chapters.

At appropriate places in the text, structure and composition, structural determination and analytical procedures (chromatography and several kinds of spectroscopy), biosynthesis and catabolism, function, and taxonomy are discussed.

F. D. Gunstone

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obituary

T. D. Lysenko

It is a paradox that Trofim Lysenko, who died at the age of 78 on November 20, 1976, was for 30 years the best known spokesman for communist science both inside and outside Russia. For he was not a member of the Communist Party, he was hardly a scientist, and of course he never left Russia. It was his part in the fluctuating relations of successive heads of the Soviet Government with science and with agriculture in the Soviet Union that aroused this world-wide interest.

What first brought Lysenko to notice was his claim in 1930, based on his work at a Ukrainian plant breeding station, that pretreatment of grain would enable Russian farmers to sow winter wheat in spring and thus to reap an increased harvest. This claim, made in the year of Stalin's 'great break', was soon linked with wider generalisations.

It was said that such treatments would change heredity, displacing the slow methods of orthodox plant breeding. Hence they would revolutionise both the practices of agriculture and the theories of biology. For sceptical observers it was not clear whether these claims were based on scientific experiment or philosophic expectation. But it was believed that they were the result of collaboration with a Marxist mentor, Professor I. I. Prezent, who for long remained in the background. Nevertheless, first scientific and then political journals in Russia began to discuss them. Eventually the newspaper *Pravda* and the whole Soviet press was seen to be accepting the evidence and propagating the arguments of Lysenko. The demand arose for a public discussion of the relative merits of Western idealistic bourgeois genetics and the new practical materialistic and Marxist science.

At this point other branches of the Government stepped in. An International Congress on Genetics which was being arranged in Moscow for 1937 by N. I. Vavilov, the President of the Lenin Academy of Agricultural Sciences, was ordered to be postponed. In its place a debate was arranged before a purely Russian and largely agricultural audience. Here the geneticists were allowed to defend themselves and there was a serious argument. But by this time Stalin's purges were in full swing and when the great man himself appeared on the platform and intervened to exclaim "Bravo, Comrade



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Lysenko" it was obvious how the argument would be settled.

Vavilov had been removed from some of his offices before the meeting. After it was over the leading geneticists, one by one, disappeared. Those who were members of the Party were shot. Vavilov himself was not arrested until 1940. He was then sentenced to death as a British spy (having been a frequent visitor to Britain). He was reprieved but later died in prison. Lysenko took over his chief posts and these he continued to hold for twenty-five years.

News of what had happened leaked out of Russia only after the war. By 1946 critics in Britain and the United States were beginning to make serious charges. Lysenko and the Soviet Government were accused of collusion in scientific fraud and political murder. Lysenko's second active phase then began. He denounced the critics abroad, and any who might support them at home, as enemies of the people.

To make it clear what this meant, a second debate was now arranged. The battle lines were laid down in advance. On the Marxist and Leninist principles of struggle and partisanship, two biologies were seen to be implacably opposed. On one side was Lysenko's own science, honest, creative and revolutionary, marxist and materialist, proletarian and patriotic. On the other side were the false and futile works of the Mendelist-Morganist Weismannists who were hardly human and almost entirely foreign.

The meeting took place in August 1948. Again it was at a time of crisis, the battle over Berlin. A few surviving supporters of genetics were allowed to speak, heckled and ridiculed from the floor. Lysenko's followers filled the hall. They also filled the pages of the proceedings with long citations from Marxist authorities. What their state-

ments amounted to in a positive sense was simple but comprehensive. It was the assertion that the inheritance of acquired characters must be the basis of all plant and animal improvement. From this immense generalisation only man was excluded, eugenics and medical genetics having been abolished in the 1930s. In a negative sense there was another conclusion which could also be put in a nutshell. As Lysenko said: "No efforts of the advocates of the chromosome theory of heredity... to lend their theory a materialistic appearance can change the character of the theory which is essentially idealistic" (*Applause*). The obstinate material character of the chromosomes and heredity, which had been unknown to Marx, was thus shown never to have existed.

The climax of the meeting came when Lysenko announced that his report had been examined and as was to be expected, approved, by the Central Committee of the Party (*Stormy Applause, Ovation, All Rise*). After Stalin's death Lysenko added the footnote that his patron had most diligently edited the report before approving it. Well he might, for the 631 pages of the Proceedings translated into many languages were ordered to be circulated in well-bound editions at home and abroad.

At the end of the meeting three professors of genetics admitted their errors and promised to follow Lysenko's guidance in future. Those whose honesty and courage forbade submission were degraded and dispersed. By a series of decrees all teaching and research in genetics, agricultural or medical, was suppressed. Books were destroyed, type broken up and libraries purged throughout the Soviet Union. There followed a period of idolisation of Lysenko. He was now a Hero of Socialist Labour with nine Orders of Lenin. His statue appeared in public places and the State Chorus sang his praises.

Lysenko's career, so far, had been sustained by a stream of projects, like his first proposal, to parallel the work of collectivisation in revolutionising Soviet agriculture. Vernalisation of wheat was followed by inherited vernalisation. Hybridisation within varieties was followed by pollination without hybridisation and hybridisation without pollination. Summer potatoes in the North were followed by spring wheat in Siberia; by a *tour de force* wheat became rye and by love-marriages

extraordinary fruit hybrids became fertile, while under the master's hand a hazel sprouted from a hornbeam.

During Stalin's lifetime the succession of claims was too rapid to disprove and too dangerous to contradict. But when he died critics raised their heads. And when Khrushchev, three years later, announced a thaw, they dared to open their mouths. The fact that none of Lysenko's claims had ever been vindicated might now, it seemed, prove to be his undoing. But matters of state are not to be so easily disentangled. As can happen even in a free world the industries of communication and education were now implicated with the establishment in a general deception. They were also infiltrated by the secret police. What was worse, it turned out that the successor, Khrushchev himself, liked Lysenko; not as Stalin did, for his theory but for his earthy language and proverbial wisdom.

So it was that Lysenko enjoyed one last spell of power. The virgin lands of Kazakhstan and the novel possibilities of cattle breeding offered him new fields to conquer. The two men embarked on these ventures in partnership and their failure seems to have contributed to the downfall of both. For Zhores Medvedev's exposure of this failure in *Samizdat* led to his confinement in a psychiatric clinic. But it had been known to the Praesidium before it deposed Khrushchev on October 14, 1964. The customary newspaper campaign followed. But now, for the first time, it was turned *against* Lysenko. He was dismissed as head of the Academy's Institute of Genetics on February 1, 1965. And the Institute itself (not for the first time) was dissolved.

News of these developments was released abroad. But the Russian public were only by rumour allowed to perceive that a great pillar of the state had somehow been withdrawn; and to feel that two systems of belief, abandoning the struggle to the death, were to be allowed to shake down together. When summer arrived, sure enough, the Academy in Moscow was allowed to celebrate the centenary of Mendel's paper and to dispatch to Brno in Czechoslovakia seventy geneticists, the largest of all delegations, to lay a wreath on the Abbot's monument.

Thus everything, it seemed, could now be amicably and democratically arranged. Lysenko, for his part, could remain a member of three academies. He could keep his titles and pension, his following of university professors, his journal regularly published and his Experiment Farm in the Lenin Hills. And the grateful geneticists were free to pick up what they could, first in medical, then in animal, and ultimately

in plant genetics, from the ruins that Lysenko had left them. Meanwhile the Government could forget that it had ever interfered in these academic matters.

Outside Russia, however, the damage that had been done to the relations between scientists and communism could never be repaired. The view of communism as meaning the liberation of science, held in the 1920s had been turned inside out. With this painful reservation, inside Russia, it is a hopeful sign that the restored geneticists are proposing to hold in Moscow in August 1977 the International Congress on Genetics so tragically abandoned forty years ago. The history, the theory, and the philosophy of genetics are not among the 35 headings proposed for discussion. But, no doubt, the names of the men whom Lysenko destroyed will be discreetly remembered.

One more question should be asked: was Lysenko a charlatan? It is an important question because fraud is always, and in all countries, bound to collect in the wide fringes of science. He was obviously ill-educated, quite shallow, very cunning and a little deranged. As Vavilov put it, privately, he was "an angry man" of a kind we all know. And he found his anger brought him great rewards. On the other hand, his followers were far from angry. They had calculated the rewards that a corrupt system had to offer them.

It remains to say that the world of science owes a debt to three bodies of men in connection with Lysenko. There were those like Vavilov who died in fulfilling their duty. There are those like Andrei Sakharov and Zhores Medvedev who resisted and survived. And there are those devoted scholars outside Russia, notably David Joravsky and Loren Graham, who have enabled us now to understand so much of what happened. C. D. Darlington

S. B. Pessôa

THE DEATH of Professor Samuel Barnsley Pessôa, removed the grand master of parasitology from Latin America, a region where the subject has flourished vigorously throughout this century. His predecessors in Brazil included H. de Beaufaire Aragão, Carlos Chagas, H. da Rocha Lima and Oswaldo Cruz. Pessôa himself founded a school of workers who have penetrated into all corners of South and Middle America. The training of the first generation stemmed from their close association with German, French and Italian scientists either directly in the New World or during their own visits to Europe in pre-world war days. Pessôa's monumental book, *Parasitologia Médica*, has gone through many editions and is in constant use in

Portuguese and Spanish speaking countries.

Pessôa was born in São Paulo on June 4, 1898, the son of Leonel Pessôa and Anna Barnsley, who was of English descent. He spent the greater part of his life in São Paulo, and died there on September 3, 1976.

After qualifying in medicine, he entered in 1925 the General Prophylactic Service of the State of São Paulo as an experimental officer attached to the Institute of Hygiene. His work took him far afield and he quickly became acquainted with the severe sanitary problems of the rural areas as well as those of the city. The distress and poverty of so much of the population transformed him into an ardent fighter for the abolition of the system, which accepted this situation with equanimity, and his ideas became increasingly revolutionary.

His abilities were quickly recognised both in the academic and in administrative fields: he was appointed to a Chair in the Faculty of Medicine of the University of São Paulo in 1931 and was later made Director General of Health. In the latter role he entirely re-organised the service by starting large scale campaigns against endemic diseases and initiating research projects. He took an active part in the latter and made many original contributions notably in muco-cutaneous leishmaniasis, trachoma and schistosomiasis. His observations on espondia and on the transmission of this form of leishmaniasis were fundamental. He studied the epidemiology of Chagas' disease and disentangled some of the problems of the cardiac complications and other phenomena of 'mega'.

Pessôa's reputation was so great that he was appointed to numerous chairs throughout Brazil and even in his last years, he was still commuting between different universities from one end (e.g. Goiás) of Brazil to the other (e.g. Santa Catarina). Pessôa never hesitated to express his views and he was often threatened with prison or dismissal. In the last 15 or 20 years of his life, he became entranced by the parasitic protozoa of snakes and lizards, working both at the Institute of Butantan and in the field on these parasites, which had always attracted Brazilian scientists; he disclosed many new species and methods of transmission by leeches and other invertebrates.

Pessôa was a colourful character, always welcome in international gatherings, where his astute and original mind was much appreciated. But above all, he was revered by his past pupils who could count both on his loyalty and protection during trials and persecutions, and invaluable advice regarding their scientific problems.

P. C. C. Garnham